Chapter 09

Epigenetics and Orthopedics

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Abstract

Especially in recent years, there are many cases in which the known genetic analyses are not enough and that can be explained with the mechanism beyond genetics, which are studied under the titles of epigenetic regulation. 'Epigenetic' term refers to activity of the hereditary phenotypic changes that are not resulted in changes in DNA sequence. The objective of this study is to provide information about the epigenetic mechanisms and some epigenetic disorders that may also involve orthopedic signs and symptoms.

Keywords

Orthopaedics; Epigenetic Disorders; Chromosomes; DNA

Introduction

Although the structure of DNA and sequencing of nucleotides are same in all cells of a living creature, intercellular differences are caused by the variations in gene expression. The mechanisms that determine how long, when and where the genes will function, and occur during the emergence of genetic information, which is coded in DNA without any change in the sequencing and structure of DNA are called epigenetic [1,2].

Epigenetic Mechanisms

Gene expression is primarily regulated by two mechanisms [3]:

1. Regulation of the activities of proteins that activate and suppress transcription.
2. Covalent modifications occurring in DNA and chromatin (epigenetic control).

A profile known as epigenotype is established with the contribution of epigenetic mechanisms, environmental factors and some other factors that have not yet been defined. Reflection of a genotype on this profile results in phenotype [3].

Epigenetic mechanisms are gathered under three main titles [4]:

1. DNA methylation,
2. Histone modifications,
3. RNA-induced silencing.

Hereditary changes occur in gene expression as a result of the combined function of these mechanisms. An error in any of these mechanisms causes excessive increase or suppression of gene expression, leading to epigenetic disorders [4].

Some Orthopedics Related Epigenetic Disorders

Epigenetic which is more unsteady than genotype is thought to underlie many diseases. The diseases that resulted from the mutations causing an erroneous epigenetic profile are known as epigenetic disorders which are studied under three main titles [5].

"Imprinted" Disorders

Genomic "imprinting" is the parents related change in the expression of a certain gene. Normally, expression variations present in the alleles from the parents, and only one allele is expressed (monoallelic expression). The disease resulted from the mutations affecting this mechanism are known as "imprinted" disorders.

Vast majority of "imprinted" genes have been shown to be associated with growth and behaviours. These genes are mostly expressed in the brain, and thus mental retardation is often seen in the phenotype [6].

These diseases develop by disruption of the allele-specific expression profile as a result of the loss or gain of DNA methylation (loss of imprinting). For example, in Beckwith Wiedemann syndrome (BWS); the expressions of maternal genes are decreased, while the expression of paternal genes are increased as a result of the various

mutations/loss of imprinting in the eight "imprinted" gene in the 11p 15.5 region [7]. This disorder manifest with organomegaly, omphalocele and macroglossia. Orthopedic findings include spastic cerebral palsy as well as hemihypertrophy. Termination of the growth in large extremities may be needed in orthopedic treatment [8].

In addition, "Uniparental Disomy (UPD)", which is caused by both 11th chromosomes to be inherited from the father leads to the same phenotype [9]. The examples for uniparental disomy are Angelman's syndrome and prader willi syndrome. In Angelman's syndrome, normally maternal allele of ubiquitin protein ligase 3 gene located on 15q11-q13 is expressed, but the expression of this allele, thus expression of the gene is suppressed due to various mutations/loss of "imprinting" in these patients [6,10].

Prader willi syndrome is caused by the partial deletion of chromosome 15. It progresses with growth retardation and hypoplastic genitalia. Hip dysplasia and juvenile onset scoliosis may occur [8].

Silver-Russell syndrome and pseudohypoparathyroidism have also been reported as the other "imprented" disorders. Typically expected 5 clinical findings in Silver-Russell syndrome (SRS) include low birth weight, short stature, characteristic facial appearance (open forehead, triangle-hypoplasic face), assymmetry in the extremities, trunk or face, and clinodactilia of the fifth finger [11].

Pseudohypoparathyroidism is a rare genetic disorders caused by the ineffectivity PTH in the target cells [8]. It is characterized with clinical and chemical features of hypoparathyroidism, and is associated with a round face; a short and thick body; short and thick fingers; short metacarpal and metatarsal bones; mental retardation and radiological calcification symptoms. It is also associated with thyroid and ovarian dysfunction.

Disorders Caused by Structural Change of Chromatin

These are the diseases resulted from the mutations of trans and cis positions that change the structure of chromatin [3].

Disorders of trans position are caused by the mutations occurring in the genes that code proteins, which are involved in the regulation of chromatin structure.

The known disorders of trans position include Rett syndrome, X related α-thalassemia/mental retardation syndrome (ATR-X), "Immunousseous dysplasia-Schimke" type Rubinstein-Taybi syndrome and methylenetetrahydrofolate reductase (MTHFR) insufficiency [7].

Rett syndrome progresses with the loss of growth stages and stereotactic hand movements. In addition, C-shaped scoliosis unresponsive to bracing is also seen. Spinal instrumentation involving entire scoliosis and kyphosis should be administered in the treatment. Furthermore, spasticity results in joint contractures and is treated as in cerebral palsy [8].

Rubinstein-Taybi syndrome (RTS) manifests with characteristic facial appearance, and wide fingers and toes. Growth retardation, delayed bone age, mental retardation, difficulty in breathing and swallowing are also seen. The other findings include multiple inherited defects such as a short stature, microcephaly, micrognathia, hearing loss, skin problems, hereditary cardiac abnormalities, and renal anomalies.

In methylenetetrahydrofolate reductase (MTHFR) insufficiency, clinical findings such as peripheral neuropathy, growth retardation, hypothonia, stroke, and thrombosis are observed [12].

Disorders of cis position manifest with the affected chromatin structure as a result of the mutations occurring in DNA. Among the disorders of cis position, Fragile X syndrome is resulted from the increased number of CGG triple repeats in the 5' end of FMR1 gene.

The symptoms of Fragile X syndrome include big ears, a long face, a wide forehead, mallocclusion (closure failure of jaws) congenital cardiac anomalies, pigeon breast, hyperextensibility in the fingers, hypotonia, diplopia, and large testes.

In addition, "locus control region (LCR)" deletion and facial scapulohumeral muscular dystrophy (FSHD) have been reported as cis position disorders.

Facial scapulohumeral muscular dystrophy is a rare disease which manifests with weakness in the facial mimick muscles and shoulder muscles and becomes prominent in puberty period.

Cancer

mIn 1983, cancer cell genomes have been shown to be hypomethylated compared with the normal cells. Transposons are activated by hypomethylation of the genomic repeat sequences, causing genomic instability and associated rearrangements. In addition, loss of methylation is known to affect severity of disease and metastasis [9]. Gene-specific hypomethylations are also seen in cancerous cells. Hypomethylation usually occurs in CpG islets, changing chromatin structure and suppressing gene expression. Hypomethylation in the promotor regions of tumor suppressor genes that are involved in cellular cycle, signal conduction pathway, DNA repair, and apoptosis suppresses the expression of these genes. This provides growth and proliferation advantage to the cancer cells, facilitating metastasis [7].

In addition to the change of global methylation profile, gain or loss of DNA methylation in "imprinted" genes also causes the development of cancer. The allele of an "imprinted" gene involved in the cellular growth and proliferation, which is normally silent can be activated by the loss of methylation, increasing expression of the gene. For example, in colon, lung, liver and ovarian cancers and in Wilm's tumor; cancer develops due to the increase in gene expression with the loss of methylation in the maternal allele of insulin growth factor-2 gene, which is normally silent [9].

In general, cancer is thought to be resulted from genetics and genetical changes such as re-arrangements, mutations, deletions and amplifications that lead to abnormal expressions of all regulatory genes and oncogenes. As is known, cancer is not limited with only genetical changes and it also involve epigenetic modifications. Accumulated evidence shows that cancer is associated autophagia, apoptosis, celllular mobility and abnormal cellular functions in DNA repair. These cellular functions are al least partly regulated by HDACs [13].

Genomic DNA surrounds highly protected histone proteins H2A, H2B, H3, and H4, in the eukaryotic cell and forms the nucleosomes that are fundamental structures of chromatin. Chromatin organization plays a key role in the control of gene expression. Epigenetic modifications can regulate hereditary or reversible gene expression without changing DNA sequence, and also can alter architecture and accessibility [14,15].

Histone acetylaston, which is an important regulator of the transcription and control of the gene is controlled by histone acetyltransferases (HATs) and histone acetylases. HATs subject lysines of histamine proteins to acetylation, causing loosening of the chromatin structure, and thus gene activation is stimulated. Conversely, histone deacetylases (HDACs) remove the acetyl groups from hyperacetylated histones and suppress overall gene transcription [13].

DNA methylation and post-translational acetylation of histones constitute two basic mechanisms that are responsible for epigenetic regulation of the gene expression [16,17]. Acetylation open the intensified chromatin structure by decreasing the affinity of DNA for histones and releasing histone tails from the binding DNA, and thus transcription factors, accessory factors and RNA polymerase II complexes are provided to access DNA. Acetylation levels are resulted from the balance between teh activities of HATs and HDACs.

HATs subject the lysines to acetylation in amino-terminal tails of rich histone proteins, causing charge neutralization and a simpler, clear and transcriptionally active chromatin structure. On contrary, HDACs are the enzymes that sometimes resist to effects of HATs by reversing the histones and separate acetyl groups from 1-N-acetyl lysine amino acids on the histones mostly by acompanying supressor gene expression. HDACs can also regulate the genes through non-histone substrates. For example, tumor suppressor p53 can directly be deacetylated by HDAC1 and SIRT1 [18].

Accumulated evidence have demonstrated that HDAC play an imortant role in various biological processes such as inflammation, cellular proliferation, apoptosis, and carcinogenesis.

HDACs have an increased expression in many cancer types including ovarian, breast, bladder and other cancers [19,20]. HDACs are thought to support occurrence of cancer through interaction with acylation and transcriptional regulators. Therefore, HDAC enzymes are defined as attractive targets for cancer treatment.

Various evidence show that HDACs play and important role in in hematological malignities. Vorinostat, the first HDAC, which will be approved by the Food and Drug Administration (FDA) has been used for treatment of patients with cutaneous T cell lymphoma [21]. Recently, more role and molecular mechanism of HDACs have been discovered in hematological malignities. Abnormal expression and activity of HDACs are frequently seen in hematological malignities. It has been shown that HDACs are highly expressed in classical Hodgkin's lymphoma (HL) of classes 1, 2 and 3, and lead to poor outcomes in HL with decreased expression of HDAC1 [22].

In their study, Gruhn et al. showed significantly high expressed HDAC1, HDAC2, and HDAC8 in all samples. A high HDAC4 expression was associated with initial leukocyte count, T cell ALL, and poor response to prednisone [23]. These data indicate that HDAC4 may serve as a drug target in childhood ALL, and especially in patients who gave poor response to prednisone.

Wang and collegues analyzed the levels of HDAC expression in benign and malignant human prostate tissue and PCa cell sequences. The results showed increased level of HDAC1-5 in these samples. Moreover, HDAC inhibitor SAHA can suppress the growth and invasion of prostate cancer [24].

The expression levels of class I HDACs (HDAC1-3) rather than class II HDACs are significantly higher in ovarian cancers compared to normal ovarian tissues. HDAC inhibitor romidpsin (FK228) can

selectively inhibit class I HDACs. Suppression of HDAC1, 2 and 3 gene expression by SiRNA suppresses the growth of ovarian cancer cells [25,26].

Studies of HDACs in bladder cancers have being conducted in recent years. Expression levels of HDAC1-3 significantly increases in bladder cancer. High-degree non-invasive papillary bladder tumors are aassociated with high levels of HDAC1 and HDAC2 [20]. In addition, expression level of HDAC4 is markedly higher in transitional cell carcinomas of the bladder compared to the normal bladder tissue [27].

Expression levels of HDAC1, 2, 3, and 7 were dedected in 170 neighboring tissues in surgically resected 170 primary hepatocellular carcinoma, and the correlations between these levels, and clinical data and patient survival. HDAC 1,2 and 3 were expressed significantly higher in cancer cells compared to the normal tissue. Expression levels of HDAC1, 2, and 3 are highly correlated with tumoral grades. A high levels of HDAC2 caused a poor outcome in low grade and early stage tumors. HDAC2 (HCC) can be a predictor of independent survival [28].

Numerous evidence have shown that HDACs plays a crucial role in regulation of colon cancer. High levels of HDAC have been reported in colon cancer. HDAC3 protein increases in colon tumors. Suppression of HDAC3 in cell sequences of colon cancer resulted in growth inhibition, decreased cellular survival, and increased apoptosis [29].

Treatment Approaches

Upon understanding of that most human diseases have epigenetic bases, medication research & development studies for correction of the epigenetic errors have gained speed. The most promising compounds among the developed drug candidates are DNMT inhibitors and HDAC inhibitors [4].

DNMT inhibitors are examined under two classes as the compounds with or without nucleoside analogue, according to the action mechanisms of DNMT inhibitors. Nucleoside analogues show a structure similar to DNA basis and are involved in the structure of the chain, which is newly synthesized during replication [5]. Covalent bonds are established between the compound involved in the DNA structure and DNMTs, and activation of the enzyme is inhibited, providing the newly synthesized chain to become hypomethylated.

HDAC inhibitors can change expression of some genes by providing histone acetylation, and can affect biological activitiess by increasing acetylation of some non-histone proteins such as transcription factors and tumor suppressor proteins. By functioning of HDAC inhibitors, deacetylation of histones is inhibited, histones remain acetyled, and transcription is maintained [30].

In vivo studies conducted with HDAC inhibitors have shown that these compounds significantly reduce tumoral growth and metastasis. DNMT and HDAC inhibitor groups that are drug candidates can be administered alone, in combination, or combined with various cytotoxic agents such as chemotherapy and radiotherapy [4,5].

Although it is thought that epigenetic disorders can be more easily treated with medication compared to genetic disorders, promising compounds for the treatment of epigenetic disorders have some disadvantages [7,31,32]. Because of the reversible nature of epigenome, effects of the compounds administered are short-termed, and the patients have to use the drugs lifelong.

Whereas DNMT and HDAC inhibitors are seen noteworthy to be tested with clinical trials because of their non-specific effects, despite they are thought to cause global hyperacetylation, deacetylation and demethylation [5].

Systemic administration of the currently available HDIs may have adverse effects on the skeleton. but these drugs may provide some benefit for orthopedic applications such as bone tissue engi-

neering and heterotopic ossification, fracture repair, osteoarthritis, and bone tumor treatment [33]. Localized HDI treatment can augment healing processes. For example, addition of the cyclic depsipeptide largazole to a macroporous biphasic calcium phosphate scaffold promoted greater bone formation than scaffold alone in rabbit calvarial bone defects. This was attributed to the induction of Runx2 and BMPs [34]. Thus it has been suggested that HDIs could be developed into adjuvant therapies to promote faster skeletal healing [35]. Hdacs may also regulate osteoarthritic phenotypes. HDIs alter the integrity of the cartilaginous extracellular matrix and OA disease progression. HDIs have the potential to protect articular cartilage by preventing matrix degradation; [36].

The antiproliferative and chemo/radio-sensitizing properties of HDIs have made them attractive epigenetic therapies for cancers. The relatively modest side effects of these drugs have led to their widespread testing in many clinical trials. Xenograft models of prostate and breast cancer bone metastases indicate that HDIs can access bone marrow and reduce tumor burden [37,38]. The *in vivo* effectiveness of HDIs against other bone tumors, such as osteosarcoma and myeloma, transplanted into mice is also an active area of investigation [39]. The ability of HDIs to kill cells from human and canine osteosarcomas, chondrosarcomas, and other primary bone tumors has been well documented by a number of *in vitro* studies [39-41].

It would be possible to clarify epigenetic profile, better understanding of the genetic mechanism, and development of new treatment facilities with the completion of the Human Epigenome Project, which has been initiated in 2003.

Conclusion

Understanding of that besides the mutations seen in genotype, the mutations causing change of epigenotype are also important in hereditary diseases, has provided the studies a new and different dimension. Studies in future will try to understand which epigenetic pathways regulate or disrupt the genes that are involved in differentiation and maturation, providing new approaches for the prevention and treatment of numerous epigenetic disorders.

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