EPIGENETICS OF SKELETAL DISEASES

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ABSTRACT

Purpose of review. Epigenetic mechanisms modify gene activity in a stable manner without altering DNA sequence. They participate in the adaptation to the environment, as well as in the pathogenesis of common complex disorders. We provide an overview of the role of epigenetic mechanisms in bone biology and pathology.

Recent findings. Extensive evidence supports the involvement of epigenetic mechanisms (DNA methylation, post-translational modifications of histone tails and noncoding RNAs) in the differentiation of bone cells and mechanotransduction. A variety of epigenetic abnormalities have been described in patients with osteoporosis, osteoarthritis, and skeletal cancers, but their actual pathogenetic roles are still unclear. A few drugs targeting epigenetic marks have been approved for neoplastic disorders and many more are being actively investigated.

Summary. Advances in the field of epigenetics underscore the complex interactions between genetic and environmental factors as determinants of osteoporosis and other common disorders. Likewise, they help to explain the mechanisms by which prenatal and postnatal external factors, from nutrition to psychological stress, impact our body and influence the risk of later disease.

KEYWORDS: Epigenetics, DNA methylation, fractures, adaptation, microRNA, histones

Introduction: Genetics, epigenetics, and adaptation

Like other animals, humans need molecular and functional stability to survive. Thus, cells must preserve their main characteristics through mitosis and cell division. Also, we need to preserve the shape and function of our body through generations. In fact, the appearance of deleterious genetic mutations is a well-known cause of disease.

However, cells and whole organisms also need to adapt to environmental conditions. Indeed, the ability to adapt to different environments is key for survival. In this sense, the body reacts to environmental factors trying to sustain or improve body functions in the context of adverse or changing conditions. However, environmental influences may also cause a number of body disturbances, either directly or as a consequence of adaptative responses. For example, astronauts experience a rapid loss of bone mass during space flights. This is an adequate response to the reduced mechanical requirements of the skeleton. However, it may cause troubles when they come back to gravity.

Physiological adaptation may involve a number of genetic and non-genetic mechanisms. Spontaneous changes in DNA sequence may, by chance, result in a survival advantage, related to a better adaptation to the environment. This is the basis of biologic evolution. These responses are persistent in time, but very slow. They usually occur through many generations. Thus, they are not suitable when short-term homeostatic changes are required. Other adaptations are mediated by non-genetic biochemical mechanisms. For example, the stress response includes a number of changes in catecholamines, glucocorticoids, and other hormones that reinforce the ability of the body to fight against the adverse environment (either internal or external) (1). Unlike genetic changes, these modifications are fast, but transitory. Somewhere between those adaptative modifications lie the epigenetic changes. They may be relatively rapid, and at the same time stable through cell divisions and perhaps through generations. In fact, as described in the following sections, epigenetic marks include a number of mechanisms, triggered by environmental influences and other stimuli, which are capable of modifying gene activity in a stable manner, without altering DNA sequence. These mechanisms play a central role in another particular process of adaptation: the differentiation of cells, which must express and repress unique sets of genes and display distinct tissue-specialized functions despite the fact that all they have an identical genome (2).

Epigenetic mechanisms

The best known epigenetic mechanisms are DNA methylation, post-translational histone modifications and non-coding RNAs (ncRNA). They act as regulators of gene activity by controlling chromatin assembly and gene transcription, or post-transcriptionally (ncRNA's) by controlling protein translation.

Bone remodelling is important for repairing bone damage and maintaining mineral homeostasis. The major cell types involved in remodelling are osteoblasts, osteoclasts and osteocytes. Bone-forming osteoblasts derive from mesenchymal stem cells (MSCs), whereas osteoclasts, the bone-resorbing cells, derive from hematopoietic precursors. Epigenetic mechanisms drive the differentiation of both the osteoblastic and the osteoclastic lineages, as well as the activity of differentiated bone cells. Some examples are mentioned below, and the reader is referred to more comprehensive reviews of this field (3–5).

In general, the methylation of gene promoters is associated with repression of gene transcription, but this is not a constant phenomenon. Indeed, methylation of enhancers and other regulatory regions may have both stimulatory and inhibitory consequences. Furthermore, the methylation-related repression of some gene inhibitors may result in enhanced expression of their target genes. Yu compared DNA methylation in osteoblasts and other cells and identified methylation patterns that were related to the expression of genes specifically involved in bone metabolism pathways, thus supporting the involvement of methylation marks in the regulation of osteogenesis (6). Other studies have implicated changes in the methylation of Wnt pathway genes and the transcription factor runx2 during osteoblast differentiation (7,8).

DNA methylation and posttranslational modifications of histone tails usually act in concert to regulate gene transcription. For example, the methylation of lysine 27 of histones 3 (H3K27) tends to be associated with methylated promoters, whereas acetylation of histone tails is usually present in regions with actively transcribed chromatin. Several studies have explored the role of these mechanisms in the differentiation of MSCs (5). Specifically, the methylation of all lysines in histone 3 (K3K4, H3K27) is important for osteoblastogenesis. In line with this concept, methyltransferases such as EZH2, seem to contribute establishing the gene expression pattern characteristic of differentiated osteoblasts (9–12). Other histone-modifying enzymes relevant for osteogenesis are the histone H4 methyltransferase *Suv420h2* (13), and the bromodomain and extra-terminal domain (BET) protein family (14).

Recently, long and small ncRNA's have received a noteworthy attention due to their ability to regulate gene expression at the transcriptional (lncRNA´s) and posttranscriptional level (small and long ncRNA's). Several studies suggest that ncRNAs are involved in osteogenic differentiation and bone pathology (15–17).

MicroRNAs (miRNAs) are small, 21-23 nucleotides-long, RNAs that inhibit the synthesis of proteins by binding to target mRNAs, which results in stopping protein synthesis or even the degradation of mRNA. A few thousand miRNAs have been identified; each one having several target genes (17).

Long-non coding RNAs are more than 200 nucleotides-long and regulate gene activity by a variety of mechanisms at both the transcriptional and post-transcriptional levels (18).

In recent years it is becoming evident that chromatin spatial organization affects gene expression. Such organization determines the accessibility of transcription factors to regulatory regions. It is controlled by specialized proteins (cohesins, condensins, CTCF, etc.) that allow the formation of "loops" which in turn permit the close contact between enhancers and their target regions in distant locations of the same chromosome or even in other chromosomes. Chromatine conformation is determined by a variety of environmental signals, DNA methylation, histone marks and other epigenetic factors. Technical developments are allowing to have a better understanding of the "chromosomal conformation signatures", a term describing the collection of DNA contacts associated with specific gene expression profiles. These signatures are being actively explored as biomarkers of disease and potential predictors of drug responses (19).

Exosomes as transmitters of epigenetic signals

Cells exchange information and interact with each other via the secretion of a variety of factors, including proteins and nucleic acids, that are secreted directly into the extracellular media or contained in vesicles. Extracellular vesicles (EVs) are cell-derived corpuscles enclosed by a lipid bilayer with a diameter between 30 and 1000 nm (20). EVs are classified as microvesicles, exosomes and apoptotic bodies. Microvesicles bud directly from the cell membrane; exosomes derive from the fusion of multivesicular endosomes (containing proteins and nucleotides) and the cell membrane. Exosomes can interact with target cells by activating cell surface receptors or by delivering their content (transcription factors, non-coding RNAs, hormones...) into the cytosol of target cells. In line with their regulatory role, exosomes have been implicated in bone biology (21).

Exosomes regulate osteoclast and osteoblast differentiation and communication by delivering a number of mediators, including miRNAs (22). In theory, exosomes might deliver miRNAs in both paracrine and endocrine ways. As an example, osteoclast-derived miR-214-3p is transported into exosomes, enters circulation and, in theory, may act at distant sites. This miRNA has an inhibitory effect on bone formation and has been associated with osteoporotic fractures (23,24). Other miRNAs are also known to modulate osteoblast differentiation (25,26). Furthermore, exosomal miRNAs may participate in muscle-bone communication. For instance, Qin recently proposed that myostatin decreases the production of miR-218-containing exosomes by osteocytes, which in turn has an inhibitory effect on osteoblasts (27). MSC-derived exosomes may also play a role in bone healing and regeneration (28,29).

Environmental influences on the osteoblastic lineage: hypoxia and mechanical loads

Mechanical loading is essential to maintain bone anabolism. Epigenetic mechanisms and several signalling pathways appear to be involved in mechanotransduction (30). Mechanical stimulation influences the methylation of a number of genes involved in intracellular signalling, including G proteins (31). On the other hand, the demethylation of the sonic hedgehog promoter is required for the loading-induced osteogenesis in mice (32). Additionally, a negative feedback loop (with the participation of the methyl-CpG-binding domain protein 2 (MBD2), a "reader" of methylated cytosines) to prevent excessive osteogenesis with loading has been suggested from experiments using the osteocytic cell line MLO-Y4 (33). Both histone deacetylases 4/5 and the miR17-92 cluster appear to be required for the periostal bone formation induced by loading, in vitro and in vivo (34,35). A few studies also suggest that non-coding RNAs may be involved in muscle-bone communication (27,36,37).

There is emerging evidence for a role of oxygen availability in skeletal cell activity. Hypoxia influences the differentiation of MSCs. The sirtuin family of histone deacetylases may be involved in the response to varying oxygen levels. For example, hypoxia induces an inflammatory response in human osteoblasts which can be attenuated epigenetically by the histone deacetylase sirtuin 6 (38). Additionally, sirtuin1 may protect osteoblasts against hipoxia (39).

The developmental origin of bone disorders

The "developmental origins of health and disease" concept proposes that when the fetus or infant is exposed to adverse environmental influences, its metabolism becomes altered in a lasting way, resulting in increased vulnerability to later disease (40). In this line, a number of studies show that fetal under-nutrition, indicated by low birth weight, may be associated not only with adverse childhood outcomes, but also with increased adult prevalence of osteoporosis, diabetes mellitus, and cardiovascular disorders (41). In several studies, including a meta-analysis, birth weight has been associated with later bone mass. In general, the association is stronger with bone mineral content (BMC) than with bone mineral density (BMD), thus suggesting that early life events have a higher influence on skeletal size than on bone volumetric density (42). Among maternal nutrients, vitamin D has received greatest attention. Maternal vitamin D status has been found to correlate with fetal development (43) and with bone mass of offspring during childhood or young adulthood (44). However, the relationship of early life growth with fractures in later life is still unclear (45).

Preliminary evidence suggests that the influence of early life environment on bone is mediated by epigenetic factors. In rodents, maternal vitamin D status influences DNA methylation state in the germline, which is transmitted to unexposed second generation (46). Also, studies in a British mother-offspring cohort found an association of the methylation levels of several genes (such as eNOS, RXRA and CDKN2A) in cord blood and bone mass at 6-9 years (47,48), but replication in other cohorts is pending.

Socioeconomic status and other social factors influence bone mass. Indeed, social deprivation during early life (both pre- and post-natal) has a negative impact on the skeleton. The mechanisms involved are likely multiple and include nutritional deficiencies, psychological stress responses and persistent low-degree inflammation (49). Those responses may be mediated, at least in part, by epigenetic mechanisms, including the methylation of genes encoding the glucocorticoid receptor and several cytokines. Those changes result in exaggerated or persistent secretion of glucocorticoids and pro-inflammatory cytokines that promote bone resorption and inhibit bone anabolism (50).

Epigenetics and osteoporosis: experimental models

A few studies have explored the role of epigenetic mechanisms in experimental models of osteoporosis, usually in ovariectomized rodents. Only scarce data are available about the changes in epigenetic marks following estrogen loss (15) and a full picture of the "osteoporotic epigenome" is still lacking. Nevertheless, a number of interventions targeting histone modifiers and readers have been explored.

The bromodomains and extra-terminal domain (BET) family include proteins that act as chromatin state readers by binding to acetylated histones and regulate gene expression. They are important for the expression of NFATC1, a master regulator of osteoclastogenesis. Consequently, pharmacological inhibition of BETs suppresses pathologic bone loss in inflammatory arthritis and post-ovariectomy models (51). Similarly, the sirtuin activator resveratrol has a beneficial effect on bone mass in ovariectomized rodents. The mechanisms involved may include the regulation of miRNA-338, which in turn targets RUNX2, a master driver of osteoblast differentiation (52). NMP is a bromodomain inhibitor that also has a positive effect on skeletal homeostais in vitro and in ovariectomized rats (53). Ezh2 (enhancer of zeste homolog 2), is methyltransferase for lysine 27 of histone 3 (H3K27). Ezh2 inactivation promotes the expression of bone-related gene regulators and Ezh2 inhibitors alleviate the loss of bone mass induced by estrogen deficiency (54).

Sulphorapheno, present in many plants, also has beneficial effects in ovariectomized mice. The mechanism of action is unclear, but may involve TET, a family of proteins participating in the conversion of methyl-cytosines into hydroxymethyl-cytosines, which may reactivate the transcription of repressed genes (55).

Epigenetics and osteoporosis: human studies

A few studies have explored the association of DNA methylation in adults with osteoporosis. Thus, an hypomethylation of Alu elements in DNA extracted from blood cells has been associated with low bone mass in postmenopausal women (56). However, it is worth emphasizing that methylation and other epigenetic marks are tissue-specific. Therefore, blood cells do not necessarily are a good index of the status of other cells more relevant to bone homeostasis. This is an important issue when critically appraising epigenetic association studies (57,58). Indeed, a recent large epigenome-wide study did not reveal consistent associations between DNA methylation in blood cells and BMD (59). However, in a genome-wide methylation screening of bone tissue samples of patients with either osteoporotic hip fractures or hip osteoarthritis, we found significant differences in the methylation of a number of genes, which were enriched in the Wnt signaling pathway and other pathways related to skeletal development (60,61). This is in part consistent with GWAS data. Indeed, several regions showing differential methylation overlap with the genes with polymorphisms associated with skeletal phenotypes in several GWAS. That is the case of several genes associated with BMD (such as A disintegrin-like and metallopeptidase with thrombospondin type 1 motif 18 -ADAMTS18-, claudin 5 -CLDN5), genes associated with bone mineral content (NK2 homeobox 2 -NKX2-2), with hip geometry (cadherin 2 -CDH2-, neuregulin 1 -NRG1) or with body shape and composition (hedgehog interacting protein -HHIP-, high mobility group AT-hook 2 -HMGA2-, zinc finger protein 678 -ZNF678-, Iroquois homeobox 2 -IRX2) (see the Catalog of published genome-wide association studies at https://www.ebi.ac.uk/gwas). Reppe et al. suggested that the methylation of SOST and DKK1 (genes encoding the Wnt pathway inhibitors sclerostin and dickkopf 1, respectively) is involved in the pathogenesis of osteoporosis (62). Since bone is a heterogeneous tissue, it is unclear which cells are actually showing the differentially-methylated regions. Nevertheless, because of their relative abundance, osteocytes and other cells in the osteoblastic lineage are appealing candidates. In fact, many cells, including mesenchymal stem cells (MSCs, the precursors of osteoblasts) suffer methylation changes with aging (63,64) that might impair their differentiation capacity. Furthermore, MSCs from osteoporotic patients show distinct methylation and gene transcription signatures (7).

Biomarkers that can be measured in accessible body fluids, such as blood or urine, are appealing from a practical point of view. Among them, miRNAs, which regulate a number of bone cell activities (reviewed in

(65)), can be measured in blood, are relatively stable, and are the focus of great attention (66). Studies of miRNA in bone samples of patients with osteoporosis and controls have produced conflicting results, with generally poor replication (67). Nevertheless, a partially replicated set of miRNAs measured in plasma has been proposed to distinguish patients with osteoporosis and/or fractures and controls (68–70). Although these are promising results, replication of the performance in other independent groups of patients is needed to confirm their role as useful biomarkers for diagnostic or prognostic purposes. The usefulness as early markers of drug response is also worth exploring.

The phenotype of a cell or an individual is the result of complex interactions between genetic and acquired factors, some of them mediated by epigenetic mechanisms (figure 1). A few examples of the interaction between genetics and epigenetics have been recently revealed in the bone field. Thus, some genetic polymorphisms associated with BMD modulate the binding of miRNAs to the regulatory regions of genes important for the skeleton, such as FGF2, RANK, osteonectin or histone deacetylases (71,72). Genetic variants located on pre-miRNA promoters may also influence the expression of miRNAs which target bone-active genes (73,74).

Epigenetics and osteoarthritis

Several candidate gene and epigenome-wide studies, have assessed epigenetic changes and their potential relation with osteoarthritis (OA) development and progression. This field has been recently reviewed (75–77) and only some findings are highlighted here.

Candidate gene studies have globally shown hypomethylation of the promoter regions and, subsequently, an increased expression of certain genes. Some genes, such as ADAMTS4, MMP3, MMP 9 and MMP 13, are involved in matrix degradation; others, such as IL 8 or IL1 b, are involved in the inflammatory response, and others are signaling or transcription factors. Demethylation and increased expression of sclerostin has also been reported in OA chondrocytes (76).

Cross-sectional genome-wide methylation studies have reported a highly variable number of differentially methylated sites. Nevertheless, there is trend for enrichment of methylated CpGs at enhancers, while promoters CpGs were depleted (77). Also, several studies pointed to transforming growth factor beta (TGFb) and fibroblast growth factor (FGF) as consistent signals.

A few studies have explored the interaction of epigenetic variation on genetic susceptibility to OA and gene transcription patterns. They showed that several established OA susceptibility loci operate as methylation quantitative trait loci (mQTL), indicating that genetic variants affect allele specific gene expression via modulation of DNA methylation in cartilage (75). However, there is no clear overlapping between regions showing differential methylation in epigenome-wide studies and genes associated with OA in GWAS. Thus, much more research is needed to elucidate the interactions between genetic and epigenetic risk factors.

A few studies have been carried out in non-cartilage OA tissues. One of them, characterizing the methylome of subchondral bone, identified that 44% of the genes differentially methylated in cartilage were also differentially methylated in subchondral bone, thus reinforcing the role of bone, and not only cartilage, in OA development. Gene ontology analysis revealed a strong TGFb signature and overrepresentation of genes involved in cytokine pathways (78).

Among the noncoding RNAs, miRNAs have been the most frequently investigated. miR-140, important for chondrogenesis and osteogenesis, is decreased in OA chondrocytes. Besides, several miRNA have been reported to be down regulated in OA cartilage with increased expression of their target genes, frequently involved in catabolic pathways. Also, there is interest in exploring the role of miRNAs released from tissues into body fluids as biomarkers in OA. Thus, circulating miR-let7e has been suggested as a potential marker of hip OA (79). However, genome-wide screening of differentially expressed miRNA shows almost no overlap between results of different studies and much more data are needed prior to introduce miRNA analyses in clinical practice.

Studies in vitro also suggest a role of histone modifying enzymes in OA. For example, the expression of type 2 collagen, the most abundant collagen in cartilage, is increased by histone acetyl transferases p300/CBP (75), whereas DOT1L, an enzyme involved in histone methylation, appears to have a protective influence on cartilage health in vitro and in vivo (80).

Epigenetics and skeletal cancer

Epigenetic changes play important roles in carcinogenesis and influence the initial steps in neoplastic transformation by altering genome stability and regulating gene expression. The detailed review of this field is out of the scope of this article, but some data illustrating the implication of epigenetic mechanisms in bone tumors follow.

Multiple myeloma (MM) is characterized by the clonal expansion of plasma cells in bone marrow. Most cases appear to be preceded by a presymptomatic stage (monoclonal gammopathy of uncertain significance, MGUS) which starts and evolves in relation to a number of genetic and epigenetic changes (81,82). A global genome-wide hypomethylation pattern is frequently found in cancer cells, and predisposes to the reactivation of transposable elements and transcription of previously silenced pro-oncogenic genes. On the other hand, specific DNA hypermethylation of tumor suppressor genes may also contribute to tumor progression. These abnormalities have been observed in many cancers and appear to be also associated with the initiation of plasma cell dyscrasias and the progression from MGUS to MM, as well as the emergence of chemotherapy resistance (81).

A number of histone modifications have been described in MM, and overexpression of Class I HDAC, particularly HDAC1, is associated with poor prognosis in MM (83). miRNAs may act as oncogenes or as tumor-suppressors depending on their target transcripts. For example, inactivation of the tumor-suppressive miR-194-2192 cluster and miR-203 which target the IGF pathway is associated with the pathogenesis of MM (84,85). Exosomes containing miRNAs have also been suggested as mediators of the influence of non-hemopoietic cells present in the marrow, such as stromal cells and adipocytes, on neoplastic and non-neoplastic plasma cells (86).

Complex interactions between genetic and epigenetic abnormalities are involved in carcinogenesis. Thus, DNA instability caused by global hypomethylation may promote the accumulation of mutations and chromosomal abnormalities, while mutations of genes coding proteins involved in maintaining the epigenetic marks may further aggravate the epigenetic aberrations (87,88).

Several oncogenic and tumor suppressor miRNAs have been reported in osteosarcoma, which is the most common primary bone malignancy (89). For instance, miR-16 inhibits cell proliferation by targeting IGF1R and the Raf1-MEK1/2-ERK1/2 pathway in osteosarcoma (90), and miR-193a-3p and miR-193a-5p, which act through down-regulation of the Rab27B and SRR genes, have been suggested as biomarkers for the diagnosis of osteosarcoma and as potential candidates for the treatment of metastases (91). Emerging evidence also suggests that some lncRNAs facilitate development and progression of osteosarcoma by influencing cell growth, invasion, metastasis and cell apoptosis (92). Abnormal gene methylation likely contributes to the neoplastic transformation of osteoblasts. In fact, whereas estrogen receptors are expressed in osteoblasts, they are absent in osteosarcoma. A recent study has shown that this is associated with increased methylation of the gene. Treatment with the demethylating agent decytabine reverses those changes and inhibits the proliferation of osteosarcoma cells transplanted into mice (93).

Chondrosarcoma (CS) accounts for more than 20% of primary bone neoplasms. Hamm et al showed that loss of DNA methylation was accompanied by an increase in invasiveness of rat chondrosarcoma cells in vitro, as well as by an increase in tumor growth in vivo. In particular, sox-2 and midline (two genes that may function in tumorigenesis) were expressed at low levels in control cells but became overexpressed upon 5-aza-2-deoxycytidine treatment (94). Silencing tumor-related genes by hypermethylation also has a

significant influence on tumorigenesis in CS via dysregulating various cell networks, including cell cycle, apoptosis, cell adherence and cell-to-cell interaction pathways (95).

The fact that epigenetic changes are generally reversible makes them an attractive therapeutic target, so, not unexpectedly, epigenetic-based therapies are being extensively studied for a variety of cancers.

Therapeutic potential and perspectives

There are a number drugs that directly target epigenetic mechanisms. For example, the DNMT inhibitors azacytidine and decitabine have been approved for the myelodisplastic syndrome. Many other molecules able to inhibit DNA methyl-transferases are being studied. On the other hand, drugs modifying histones, including inhibitors of histone acetyltransferases (such as curcumin), histone deacetylases, histone methyltransferases and histone demethylases are being studied (96).

In vitro assays and animal models revealed that several histone deacetylase inhibitors have beneficial effects in the regulation of bone remodeling. They promote osteoblast activity and suppress osteoclast resorption by interfering the RANKL pathway (97).

Sirtuins are a class of enzymes with histone deacetylase activity. A number of activators, including resveratrol, are under study. Resveratrol has an anabolic effect on bone tissue and in animal models of osteoporosis. The effect may involve the interaction with SOST and FOXO genes (98).

The bromo and extra terminal (BET) family include several proteins that bind to the acetylated lysine of histones (some members also recognize other histone modifications, such as butylation or crotonylation) and have been associated with several neoplastic and non-neoplastic disorders. BET proteins act as a scaffold for molecular complexes that regulate the accessibility of transcription factors to chromatin. Additionally, BETs interact with other non-histone acetylated proteins, in particular transcription factors, modulating their transcriptional activity (99). BET-inhibiting drugs are being tested for atherosclerosis and several types of cancers. They have also shown beneficial effects in a mouse model of post-ovariectomy osteoporosis, with complex effects on both the osteoblast and the osteoclast lineages (14).

These compounds targeting epigenetic marks display some promising effects and may likely find a place in the therapy of disorders, such as cancer, in which the target tissue has a very abnormal rate of cell proliferation in comparison with normal tissues. However, the lack of genomic specificity is a considerable limitation for treating non-neoplastic disorders. Hence, compounds targeting specifically disease-driving genes are needed for the sake of efficacy and safety. In this line, the so-called SAHA-PIPs are a novel class of epigenetically active small molecules created by conjugating selective DNA binding pyrrole-imidazole polyamides (PIPs) with the histone deacetylase inhibitor SAHA. They appear to have some degree of selectivity and modulate the transcription of certain clusters of genes (96). Furthermore, the development of new epigenetic tests and international collaborations building large molecular libraries may help to carry out high-throughput screening of thousands of molecules, and, luckily, some of them may show specificity for bone-active targets.

Another approach which, although less developed so far, may be more promising in the future due to its target selectivity, is the use of RNA inhibitors, based on mimicking or inhibiting miRNAs. They may have a role in treating systemic skeletal disorders. In fact, the efficacy of small RNA inhibitors has already confirmed in other non-neoplastic diseases. RNA-based therapies might be particularly useful in local disorders and regenerative procedures. In fact, miRNA-related molecules have shown some efficacy in preclinical models of fracture and bone defects. In some cases, they were bound to inert scaffolds, whereas other researchers have tested if miRNA transfection potentiates the regenerative capacity of MSCs (100).

Conclusion

As summarized in this review, there is considerable evidence for the role of epigenetics mechanisms in bone biology, and specifically in the differentiation of bone cells. However, their actual pathogenetic role in osteoporosis and other skeletal disorders is still unclear (table 1).

Advances in this field may uncover new therapeutic targets by identifying novel genes that play a role in bone pathophysiology, whose function can be later modified to improve skeletal health. Such gene modulation may take place through the direct modulation of epigenetic mechanisms, or by other means, such as small molecules or antibodies.

Advances in the field of epigenetics underscore the complex interactions between genetic and environmental factors as determinants of osteoporosis and other common disorders. Likewise, they help to explain the mechanisms by which a variety of external factors, from nutrition to psychological stress, impact our body. Although the intergenerational heritability of the epigenetic modifications is still unclear in mammals, a growing body of evidence supports that DNA methylation and other epigenetic marks driven by the prenatal environment have an important influence on the risk of disease in later life.

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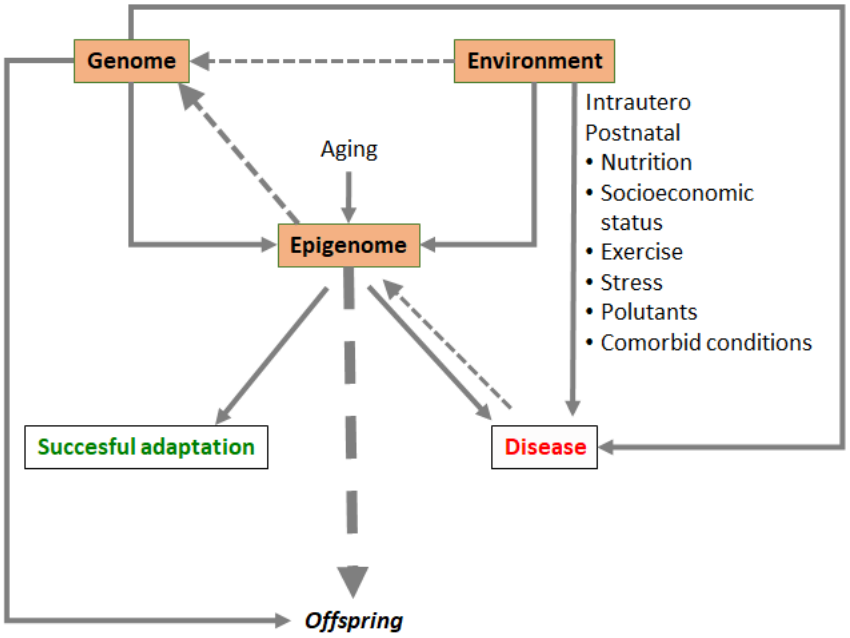
Table 1. Highlights of the results of epigenetic studies in patients with skeletal disorders.

- Several genes of the Wnt and other developmental pathways are differentially methylated in osteoporotic bone
- Some circulating miRNAs may have a role as biomarkers in osteoporosis
- Genes related to matrix degradation, and others encoding inflammatory cytokines and TGFb show specific methylation marks in the joints of patients with osteoarthritis
- There is a trend for overall hypomethylation in cancer cells, with specific hypermethylation of the promoters of tumor supresor genes
- Bone cancer cells show abnormal miRNA signatures

FIGURE 1. Diagram showing that the epigenome is determined by a variety of factors, both genetic and environmental. Epigenomic mechanisms may help cells to adapt to a changing environment, but it may also cause deleterious phenomena that eventually result in disease. Both pre- and postnatal environmental factors influence the epigenome. Of course, genetic abnormalities and some environmental factors may have a negative impact on health independently of epigenomic changes. A common issue in clinical epigenomics studies is to establish of the epigenomic changes are cause or consequence of the disease.



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