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PERSPECTIVES

The Epigenetic Regulation of Bone Mass

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Abstract

Osteoporosis is a major cause of morbidity and mortality through its association with age-related fractures. Evidence is growing that peak bone mass is an important contributor to bone strength during later life and is influenced by many factors, some of which may be modifiable during childhood. In addition, evidence has accrued that fracture risk might be programmed during intrauterine life, although the mechanisms initiating these responses remain unclear. Emerging evidence has strongly suggested that epigenetic mechanisms, such as DNA methylation and histone modification, may underlie the process of developmental plasticity. Numerous studies in animal models have shown that during embryonic and fetal development, maternal or environmental factors can disrupt patterns of DNA methylation. For example, the embryos of pregnant rats fed a low-protein diet during the pre-implantation period of pregnancy showed altered development in multiple organ systems. This dysregulation of developmental programming via abnormal DNA methylation may permit specific genes to undergo inappropriate expression during adult life, resulting in disease development. This review will summarize the relationship between developmental plasticity and osteoporosis and will focus upon the possible mechanisms by which the epigenetic regulation of bone mass may occur using two models: Maternal vitamin D status and placental calcium transfer, and the hypothalamic-pituitary-adrenal axis. *IBMS BoneKEy*. 2010 Feb;7(2):54-62.

Introduction

Osteoporosis is a skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (1). It is a widespread condition, often unrecognized in clinical practice, which may have devastating health consequences through its association with fragility fractures. These fractures typically occur at the hip, spine, and the wrist. It has been estimated that, at age 50, the remaining lifetime risk of fracture at one of these sites is 50% among women and 20% among men (2). Osteoporotic fracture has a huge impact economically, in addition to its effect on health. Osteoporotic fracture costs the United States approximately $17.9 billion/year, with the cost in the United Kingdom being £1.7 billion (3).

Although most effort in fracture prevention has been directed at retarding the rate of age-related bone loss and reducing the frequency and severity of trauma among elderly people, evidence is growing that peak bone mass is an important contributor to bone strength during later life (4). Many factors influence the accumulation of bone mineral during childhood and adolescence, including heredity, gender, diet, physical activity, endocrine status, and sporadic risk factors such as cigarette smoking (5). In addition to these modifiable factors during childhood, evidence has also accrued that fracture risk might be programmed during intrauterine life. Epidemiological studies have demonstrated a relationship between birth weight, weight in infancy, and adult bone mass. Maternal smoking, diet (particularly vitamin D deficiency), and physical activity also appear to modulate bone mineral acquisition during intrauterine life (6-8); furthermore, both low birth size and poor childhood growth are directly linked to the later risk of hip fracture (9). Many lines of evidence, including epidemiological studies and data from extensive clinical and experimental work, indicate that early life events play a powerful

role in influencing later susceptibility to certain chronic diseases, such as osteoporosis; however the mechanisms initiating these responses remain unclear. Recent data have strongly suggested that epigenetic processes are responsible for tissue-specific gene expression during differentiation and may play a key role in adaptive responses to nutritional and environmental factors during fetal and neonatal life. Thus epigenetic mechanisms may underlie the processes of developmental plasticity.

This review will summarize the relationship between developmental plasticity and osteoporosis and will focus upon the possible mechanisms by which the epigenetic regulation of bone mass may occur using two models: Maternal vitamin D status and placental calcium transfer, and the hypothalamic-pituitary-adrenal axis.

Developmental Plasticity and the Developmental Origins of Osteoporotic Fracture

Environmental influences during childhood and puberty have been shown to benefit bone mineral accrual, but the relatively rapid rate of mineral gain during intrauterine and early postnatal life, coupled with the plasticity of skeletal development *in utero*, offer the possibility of profound interactions between the genome and early environment at this stage in the life course. There is a strong biological basis for such a model of disease pathogenesis. Experimentalists have repeatedly demonstrated that alterations to the diet of pregnant animals can produce lasting changes in the offspring's physiology and metabolism. This is one example of a ubiquitous phenomenon: Developmental plasticity, that is, the ability of a single genotype to give rise to several different phenotypes, thus allowing the organism to adapt future generations to prevailing environmental conditions. In humans the importance of the intrauterine environment was initially demonstrated with associations between birth weight and blood pressure, lipid levels, and diabetes later in life. This phenomenon was termed "programming" and defined as "persisting changes in structure and function caused by adverse environmental influences at a critical stage of early development" (10;11). Epidemiological evidence that the risk of osteoporosis might be modified by the intrauterine and early postnatal environment has emerged from two groups of studies: Firstly, retrospective cohort studies in which bone mineral measurements are undertaken, and in which fracture risk is ascertained, among adults whose detailed birth and/or childhood records have been preserved; and secondly, mother-offspring cohorts relating the nutrition, body build and lifestyle of pregnant women to the bone mass of their offspring.

Thus studies in mother-offspring cohorts have shown that body composition, lifestyle and physical activity of mothers during pregnancy influence the bone mass of their offspring (6;7). Studies linking levels of venous umbilical cord IGF-1 and leptin to neonatal bone mass suggest that these hormones may be key physiological players in these relationships (12;13). Another key maternal determinant of intrauterine bone mineral accrual and offspring postnatal growth trajectory may be circulating 25(OH)-vitamin D status in pregnancy. Thus, in a cohort of 198 mother-offspring pairs, those children of mothers who were deficient (<10 ng/ml, 18% of cohort) in circulating 25(OH)-vitamin D in pregnancy had reduced whole body BMC and BMD at 9 years of age compared to those children of mothers who were replete in vitamin D (8). This relationship appeared to be mediated in part by reduced umbilical cord venous corrected calcium. Similar results relating maternal vitamin D deficiency to offspring bone mass were found for neonates in the Southampton Women's Survey cohort (14). These findings suggested that vitamin D supplementation of pregnant women, especially during winter months, could lead to long-lasting reductions in the risk of osteoporotic fracture in the offspring.

Epigenetic Mechanisms

Epigenetics refers to changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. These changes are stable and heritable, and may last through multiple

generations (15). One manifestation of epigenetic change is genomic imprinting, the process by which the expression of certain genes is determined by the gender of the parent who contributed the allele (16). Thus, through silencing of one set of alleles depending on parental origin, patterns of gene expression can be inherited without changes in the sequence of genomic DNA. In the animal world, imprinted genes are frequently involved in fetal and placental growth. Imprinting is mediated by allele-specific DNA methylation of imprinting control regions, although the precise mechanism remains unclear. Disease resulting from dysregulation of imprinting is rare but well-recognized, for example Beckwith-Wiedemann syndrome (16).

The two most studied forms of epigenetic marking are DNA methylation and histone modification. DNA methylation involves the addition of a methyl group to cytosine residues at the carbon-5 position of CpG dinucleotides. DNA methylation is generally associated with gene repression, either by decreased binding of transcription factors or by attracting methyl-CpG-binding proteins that act as transcriptional repressors (16;17). There is usually an inverse relationship between the extent of DNA methylation of regulatory CpGs and gene expression. Histone modification refers to post-translational modification of histone tails. Histones are small proteins involved in packaging of DNA into chromatin and if the way that DNA is wrapped around the histones changes, gene expression can also change. Histone modification can occur either by methylation or acetylation. These two types of epigenetic modification are mechanistically linked and work togther to affect chromatin packaging, which in turn determines which gene or gene set is transcribed. The enzymes controlling these processes have recently been indentified and include DNA methyltransferases (18).

DNA methylation patterns differ through the phases of development. After conception, and with the exception of imprinted genes, gamete methylation patterns are erased during early blastocyst formation. During the implantation stage, methylation patterns become established via *de novo* methylation by the activities of DNA methyltransferases (Dnmt) 3a and 3b. Patterns of DNA methylation are maintained through mitosis by Dnmt1 activity (19). In adulthood, there are variations in the amount and pattern of methylation depending upon cell and tissue type. During embryonic and fetal development, maternal or environmental factors can disrupt these patterns of DNA methylation; examples of this process have been shown in animal models and will be discussed in further detail in this review. This dysregulation of developmental programming via abnormal DNA methylation may permit specific genes to undergo inappropriate expression during adult life, resulting in disease development (20). Emerging evidence strongly suggests that these epigenetic mechanisms underlie the processes of developmental plasticity.

Experimental Data from Animal Models

Numerous studies in animals involving prenatal nutrient imbalance have provided important information regarding the biologic basis for developmental plasticity. For example, the embryos of pregnant rats fed a low-protein diet during the pre-implantation period of pregnancy showed altered development in multiple organ systems (20). In addition, if the pregnancies progressed to term, then the offspring had reduced birth weight, relatively increased postnatal growth and adult-onset hypertension. Further studies have shown that the administration of glucocorticoids to pregnant rats at specific points during gestation can cause hypertension and insulin resistance in the offspring in later life and can also lead to increased sensitivity to postnatal stress (21-22). Postnatal stress in rat models has been shown to induce neurodevelopmental changes in the rat pups and this leads to excessive responses to stress in later life. These changes may be mediated in part by effects on glucocorticoid receptor (GR) gene expression in the brains of the offspring (23).

Further exploratory work in this area has shown that maternal dietary protein restriction in rats leads specifically to a decrease in the methylation status of the GR and peroxisomal proliferator-activated

receptor α (PPARα) in the liver of the offspring after weaning (24). These genes are of particular interest because alterations in their expression are associated with disturbances in cardiovascular and metabolic control in animals and humans. The hypomethylation of the GR and PPARα persisted after weaning, when direct influence of the maternal dietary restriction had ceased, suggesting stable modification to the epigenetic regulation of the expression of these transcription factors. In addition, supplementation of the restricted diet with folic acid prevented hypomethylation of GR and PPARα, and the associated increase in their expression. This observation suggests that the change in DNA methylation may reflect the impaired supply of folic acid from the mother and raises the possibility of therapeutic strategies to prevent or reduce the effects of environmental insults in early life. Other studies have shown similar epigenetic changes in p53 in the kidney and the angiotensin II type 1b receptor in the adrenal gland (25;26).

This animal work is complemented by data from human cohorts and pharmaceutical studies. Thus levels of circulating cortisol predict lumbar spine bone mass in older adults and influence and rate of bone loss (27). In a study of older men and women in the Netherlands, higher cortisol levels were associated with lower femoral neck BMD in women. Additionally, different GR polymorphisms were associated with different levels of circulating cortisol (28). PPARα is involved in embryogenesis, but in the adult has a role in triglyceride metabolism, being the target for fibrate drugs. Few data exist in relation to bone disease but one recent epidemiological study examined the risk of fracture in patients taking fibrates compared with controls and found no difference (29). In contrast, thiazolidinediones, which increase insulin sensitivity through their action on hepatic PPARγ receptors, have been shown to increase fracture risk in several trials and observational studies (30). However, this isoform was not found to be hypomethylated in response to a maternal low protein diet in rats (24).

These studies show that the effects of maternal nutrition and behavior appear to target the promoter regions of specific genes rather than being associated with a global change in DNA methylation. This observation provides important clues for further work to explore epigenetic mechanisms in humans.

Epigenetics in Human Disease

Epigenetic mechanisms, including DNA methylation and histone modifications, are now well-established in the development and progression of a variety of cancer types including prostate cancer, lymphoma, head and neck, breast and ovarian cancer (31). Data in other human diseases are limited, particularly in relation to developmental plasticity and the musculoskeletal system. The first example of an association between a periconceptual exposure and DNA methylation in humans was shown in Dutch subjects prenatally exposed to famine during the Dutch Hunger Winter in 1944-1945. Exposed subjects showed persistent epigenetic differences in insulin-like growth factor II (IGF2) gene 6 decades later compared to their unexposed, same-sex siblings (32). IGF2 is known to be a key factor in human growth and development. This study further supports the importance of investigating how early epigenetic modification of gene expression may influence long-term health and disease.

Maternal Vitamin D Status and Placental Calcium Transfer

The key nutrients likely to influence fetal bone development include calcium and vitamin D; this axis, therefore, provides a model for investigating the epigenetic regulation of bone mass. The human fetus requires a total of around 30 g of calcium for bone development, most of which is acquired during the third trimester via active transport across the placenta, a process that results in greater calcium concentration in fetal than maternal plasma (33). Fetal calcium needs are primarily met by increased maternal intestinal calcium absorption during pregnancy and therefore very low maternal calcium intakes may be a risk for lower bone mass in neonates. In

addition, the importance of maternal vitamin D status has been highlighted earlier in this review. The mechanism underlying the association between maternal vitamin D, umbilical cord calcium concentration and offspring bone mass is unclear but is an area of ongoing research. Vitamin D mediates its effects by first binding to the vitamin D receptor (VDR), then by binding to the retinoic acid receptor (RXR), forming a heterodimer (34). This heterodimer then acts upon vitamin D response elements in target genes initiating gene transcription by either up-regulating or down-regulating gene products. Vitamin D response elements are DNA sequences found in the promoter region of vitamin D-regulated genes (34). Very few studies have examined methylation of the VDR or other vitamin D-related genes in human placentas. VDR gene expression was found to be repressed by epigenetic mechanisms in choriocarcinoma cells lines (35), but no methylation of the VDR was found in another study of normal placentas. However, in another recent study, 24-hydroxylase gene expression was down-regulated by promoter methylation; vitamin D response elements in calcium transporters were not studied (36).

Data from a human mother-offspring cohort have demonstrated that the expression of a placental calcium transporter (PMCA3) gene predicts neonatal whole body BMC (37). Modified expression of the genes encoding placental calcium transporters, by epigenetic regulation, might represent the means whereby maternal vitamin D status could influence bone mineral accrual in the neonate (Fig. 1). Since the effects of maternal nutrition and behavior seem to target the promoter region of specific genes rather than being associated with global changes in DNA methylation, investigating CpGs located within the promoter region of these genes, particularly those within or located near to vitamin D response elements, may provide further clues regarding the epigenetic regulation of bone mass. In addition, if validated, these epigenetic markers might provide risk assessment tools with which to target early interventions to individuals at greatest future risk.

The Hypothalamic-Pituitary-Adrenal Axis

Maternal stress is known to influence the developing hypothalamic-pituitary-adrenal (HPA) axis in the fetus. Thus, epidemiological studies have demonstrated an inverse association between birth weight and fasting plasma cortisol. Indices of the circulating cortisol profile in adult life have also been shown to influence bone density and rates of bone loss (27). As previously discussed in this review, animal studies have confirmed that protein restriction during mid and late pregnancy is associated with reduced methylation of key CpG-rich islands in the promoter region of the gene for the GR, and this results in elevated GR expression, and features of hypercortisolism (24). Further work in rats, and subsequent replication of the work in human umbilical cords, has shown that induction in the offspring of altered epigenetic regulation of the hepatic GR promoter may be due to reduced DNA methyltransferase 1 (Dnmt1) expression (38). Previous work has shown that patterns of DNA methylation are maintained through mitosis by Dnmt1 activity and in addition, the phenotype of an embryo can be modified by manipulation of Dnmt1 expression, and hence the pattern of DNA methylation (19;39). Thus epigenetic modulation of the HPA axis may represent a second mechanism for transduction between a poor maternal environment and impaired bone mineral accrual in the offspring.

Conclusions

Osteoporosis constitutes a major public health problem through its association with fragility fractures. Evidence is growing that peak bone mass is an important contributor to bone strength during later life and is influenced by many factors, some of which may be modifiable during childhood. In addition, evidence has also accrued that fracture risk might be programmed during intrauterine life. Epigenetic processes are important mechanisms that underpin developmental plasticity, and environmental factors including maternal stress and nutritional state are known to affect the long-term epigenetic state of a number of genes

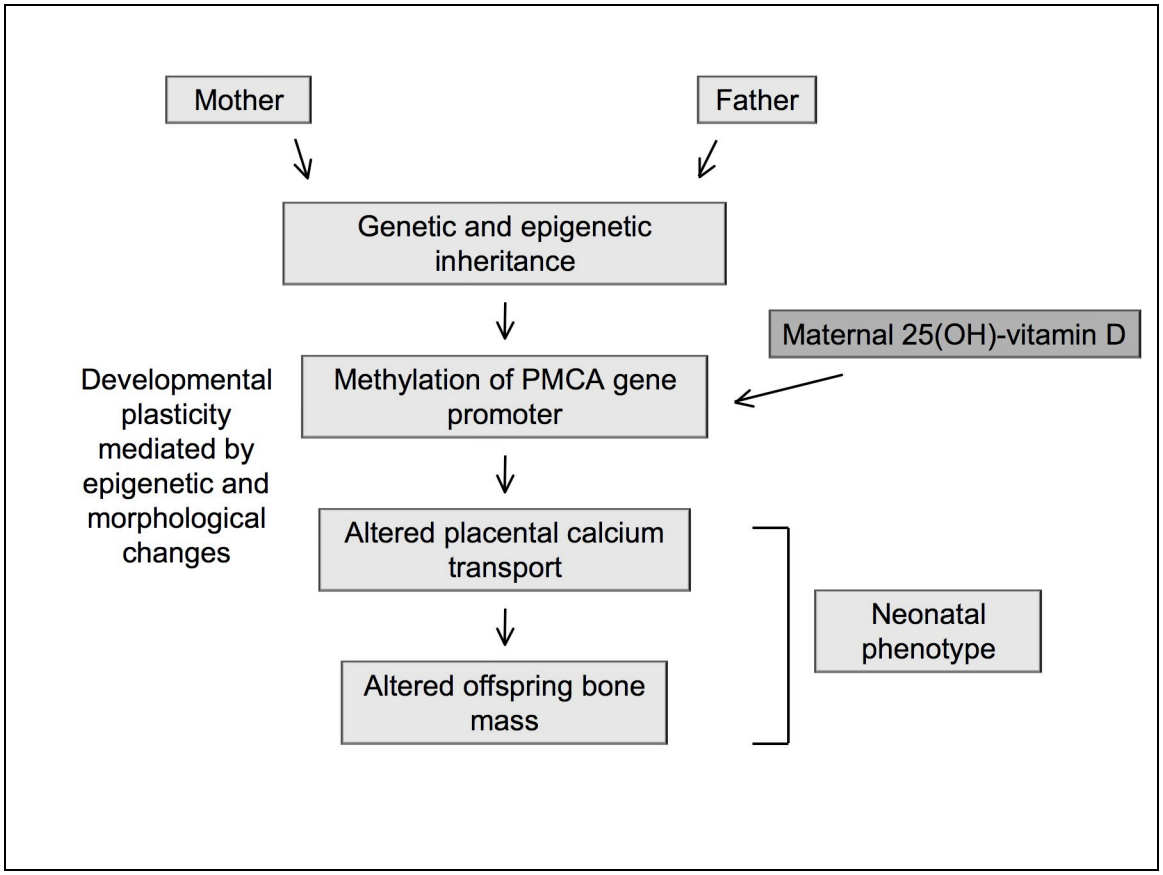

Fig. 1. Proposed pathway for epigenetic modulation of placental calcium transporter by maternal 25(OH)-vitamin D.

during embryonic and fetal development. Animal models provide a useful resource for the investigation of epigenetic mechanisms, allowing modulation of factors such as maternal diet, and accurate measurement of offspring phenotype. However, data for humans are urgently needed and it is unclear, at the present time, to what extent changes seen in animals will be replicated in human systems. Genome-wide scanning technology may provide an efficient way of identifying relevant loci in human studies, but given the limited resolution (single CpG site methylation may be missed), detailed examination of candidate genes will be required. If validated, these epigenetic markers might provide risk assessment tools with which to target early lifestyle interventions to individuals at greatest future risk.

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