Analysis of epigenetic modifications in bone cells: osteoblasts are osteoblasts isolated from bone a good model to study changes in DNA methylation?

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Epigenetics is the study of the mechanisms which regulate gene expression in a stable and hereditary way, but without altering the DNA sequence[1]. This field of research has gained importance in recent years and it is postulated that it may explain the process of differentiation of bone cells, the appearance of bone metabolic diseases, as well the inheritability of certain pathologies (for recent reviews, see[1-3]). Epigenetic mechanisms include post-translational modification in histones, regulation of protein synthesis by means of microRNA and DNA methylation.

Recently, it has been proposed that changes in levels of methylation of genes may alter the differentiation of osteoblasts and osteoclast precursors in bone tissue. For example, the transcription factors osterix and DLX5, estrogen receptor α, as well as osteopontin, are co-regulated through the methylation of DNA[4-6]. Moreover, levels of DNA methylation may be regulated by mechanical stimuli, as is the case with the promoter of osteopontin[6], suggesting that some of the effects of mechanical stimulation are due to the regulation of gene expression by epigenetic mechanisms. It should be mentioned that the methylation of the promoter regions of DNA in certain genes may regulate their expression at different stages of cell differentiation. Such is the case with alkaline phosphatase and sclerostin[7-9]. While the degree of methylation in the promoter region of alkaline phosphatase increases as the osteoblast line cells are differentiated, leading to the silencing of its expression in the osteocytes, the opposite occurs with sclerostin, whose promoter is methylated in the osteoblasts and is demethylated in the osteocytes.

Similarly to osteoblast differentiation, the genes which code for RANKL and OPG are also regulated by their levels of methylation[10], thus affecting the generation of osteoclasts, a process which depends on the relative expression of RANKL and OPG[11]. As occurs with the osteoblast lineage cells, changes in the levels of methylation of DNA also accompany the differentiation of the osteoclast precursors. This results in differences in levels of expression of genes fundamental to osteoclast function such as cathepsin K and tartrate-resistant acid phosphatase[12]. It has also been suggested that aberrant patterns of methylation of DNA may cause pathologies in which there are alteration in bone metabolism. For example, a reduction in levels of methyltransferase DNMT1, an enzyme involved in the maintenance of genome methylation, results in loss of bone mass[13]. Similarly, changes in gene expression in the chondrocytes are associated with changes in the methylation of DNA (for example, in the gene for type x collagen or of various metalloproteins in the matrix[14,15]) could be contributing to the generation of osteoarthritis.

This work is a continuation of earlier studies from the same group in which were demonstrated the role of DNA methylation in the expression of the osteocyte protein sclerostin[9], the marker for bone formation alkaline phosphatase[7], and of the cytokines involved in the generation of the RANKL/OPG osteoclasts[10]. In the manuscript of Delgado-Calle et al.[16] they explore and compare the presence of methylated CpG in purified DNA from human bone and from primary osteoblast cultures obtained also from patients with osteoporotic fractures or arthritis. The authors analysed the levels of methylation in the bone and in the

cultured osteoblasts and found a similar pattern in terms of the average level of methylation, both if all the loci were analysed or only those related to bone. Consistently, a fraction of the genes analysed deviated from the general relationship. In particular, the list of genes related with bone metabolism and which are found to be differentially methylated in preparations of bone and cultivated cells includes the receptor for parathyroid hormone, members of the Wnt and TGFβ pathway stimulation chain, and interleukins and chemokines. Modifications in the expression of these genes could have profound effects on the maturation, proliferation and survival of bone cells. However, the composition of the cells present in the bone, and the fact that the cells have been cultivated in one or two passages, should be taken into account. In particular, the majority of the cells in bone are osteocytes, not osteoblasts. Furthermore, the presence of bone marrow cells in the fragments of bone may also confuse the results. Another factor which may explain the differences found is the fact that the osteoblast cells were exposed to an artificial medium, and in an incubator. Although promising, the result reported in this work need to be complemented by more detailed studies, separating the osteoblasts from the osteocytes to evaluate the contribution of each cell population in the methylated loci.

In summary, the work of Delgado-Calle et al. demonstrates that the population of methylated genes in bone cells varies depending on the source of the material. The conclusions of the study should be treated with caution due to the difference in the types of cells present in the bone, compared with primary cultures, and the small number of replicas. However, it shows the importance of corroborating the results obtained in cell cultures with animal studies or with human samples.

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