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Molecular Insights into Prostate Cancer-Bone Crosstalk: Role of miRNA-135b

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Prostate cancer (PCa) is the second most common malignant disease in men and the fifth leading cause of cancer-related death in the world. In Spain, the incidence in 2020, according to the *Asociación Española Contra el Cáncer* (AECC) and *Asociación de Cáncer de Próstata (*ANCAP) was 33,341 cases, of which 6,112 people died because of this cancer. These statistics represent a progressive growth of accumulated cases in the last 5 years due to the increase in population ageing. In early and localized stages, PCa has a 5-year survival rate of over 98% however, survival drops substantially to 30% as the illness advances due to around 80–90% of patients with advanced PCa have metastases, particularly in bone tissue.

The generation of bone metastases is a complex process involving multiple biological and molecular pathways which leads, in the case of bone metastases generated by PCa, to an increase in bone mass at the radiographic level due to the induction of ectopic bone formation by osteoblasts. Although in PCa the bone metastases generated are prototypically osteoblastic, there is histological evidence that bone lesions are mixed, and the generation of osteolytic lesions by osteoclasts is necessary for subsequent tumor growth. This is consistent with the existing physiological coupling for bone remodeling where there is a cross-talk between osteoblasts and osteoclasts.

This remodeling is affected during the generation of metastases and triggers a vicious circle, a multidirectional interaction where the bone microenvironment formed by osteoclasts and osteoblasts plays an important role altering the bone structure and promoting the growth of PCa cells. With this in mind, our group generated a model with high penetrance to metastasize to bone using intracardiac injection of PC3 luciferase-expressing cells. The new metastatic subclone named PC3-BM, isolated from the third *in vivo* round, presented a higher incidence of metastasis to long bones compared to the parental PC3 cell line. Having this useful tool to unveil novel bone metastasis-promoting factors, PC3-BM cells were molecularly characterized leading us to identify miR-135b as a promising molecule with a relevant role in bone metastasis. Thus, the main objective is to study the role of miR-135b in those PCa cells able to disseminate and stablish metastasis into the bone and further elucidate molecular crosstalk between PCa and bone with the ultimate goal of finding predictive biomarkers of poor prognosis and new and more efficient therapies for the treatment of metastatic PCa patients. For this purpose, a Gene Set Enrichment Analysis (GSEA) of differentially expressed genes between PC3-BM and parental cell line was performed, as well as of miR-135b predicted target genes obtained by using different algorithms into miRWalk platform. On the other hand, we analyzed the effect of transient inhibition and transient overexpression of miRNA-135b in PC3p and PC3-BM cells respectively on the osteoclast and osteoblast differentiation by conditioned media assays. Expression of the most representative genes associated with bone remodeling were analyzed by RT-qPCR. The bioinformatic analysis showed in PC3-BM an enrichment of pathways associated with cancer metastasis and specifically with bone related process such as activation of osteoclasts and bone resorption or cytokine-cytokine interaction. Furthermore, among the targets of miR-135b we found an enrichment of genes related to osteoclast differentiation. Regarding *in vitro* assays, it was observed that after treating preosteoclasts (RAW264.7) and osteoblast (hFOB 1.19) with the conditioned medium from those PCa cells overexpressing the miR-135b, some osteoclastogenic genes such as CFS1, RANK or TRAP or genes related to osteoblast differentiation such as COL1A1 were significantly downregulated. On the other hand, the opposite effect was observed due to miRNA inhibition. Our results suggests that the loss of miR-135b in PCa cells may have a relevant role in bone remodeling favoring the establishment of metastasis. Therefore, this miRNA and its targets could lead us to define new therapeutic strategies to prevent or treat patients with advanced PCa more effectively and improve their clinical outcome.