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miR-135b and miR-19a as regulators for bone selection as metastatic niche in prostate cancer

Mireia Olivan [1] , Marta Garcia[1] , Leticia Suárez[1] , Marc Guiu[2] , Roger Gomis[2] , Joan Morote[3] , Anna Santamaria[1]

1) Cell Cycle and Cancer Laboratory, Group of Biomedical Research in Gynaecology, Vall d'Hebron Research Institute (VHIR) and Universitat Autònoma de Barcelona (UAB), Barcelona, Spain 2) Oncology Program, Institute for Research in Biomedicine (IRB-Barcelona) 3) Department of Urology, Vall d'Hebron University Hospital, Barcelona, Spain

Introduction: Prostate cancer (PCa) is the most commonly diagnosed neoplasm among men and the third cause of cancer-related deaths. During the disease progression, bone is the most common site of dissemination, where up to 80% of the metastases are found. About 70% of advanced stage PCa patients will experience bone metastasis, which causes bone pain, pathological skeletal fractures, spinal cord compression or hypercalcemia. These complications severely affect the patients\' quality of life and unfortunately nowadays, the detection of bone metastases indicates progression to lethality.

Aim: Therefore,the identification of molecules that play a critical role in the progression of PCa in bone will provide promising targets to develop new therapeutic strategies for a more efficient treatment of poor prognosis PCa patients.

Methods: With this purpose, a PCa cell populationwith increased bone metastases preference was generated byinjecting intracardiacally humanPCa cells (PC3 cell line) stably transfected with luciferase/green fluorescent protein in nude mice. Cells from bone metastasis were isolated and re-injected again in a new set of mice. After three rounds of *in vivo*selection, a cell population with increased bone metastatic potential was obtained (PC3-BM). Both cell populations were compared by genomic high-throughput analysis at RNA and miRNA level. On the other hand, the identification of the most relevant pathways and biological processes through anenrichment analysis of the differentially expressed genes was performed. Candidates genes were analyzed *in silico*using PCa public databases to verify their relevance in human disease. Furthermore, the integration of genes and miRNAs expression data was performed and the most promising candidates were validated by RT-qPCR and western blot. Finally, in order to study the potential role of the selected molecules in the dissemination to the bone, a transwell migration assay was designed based on co-culture experiments with osteoblast cells (hFOB or MC3T3). This assay was performed using genetically modified PC3 cells that overexpressed the candidates thought miRNA-mimics transient transfection.

Results: After the enrichment analysis 215 processes have been found to be up-regulated in the PC3-BM versus parental PCa cell line whereas 77 were downregulated (considering an FDR<0.05). Particularly, cancer/metastasis and bone-related processes are largely represented among the up-regulated pathways. Additional up-regulated enriched processes are also cell division, glucose metabolism, cholesterol metabolism, angiogenesis, and immune system-associated processes. On the other hand, DNA biology, cell proliferation and mitochondrion biology-related process were down-regulated. Out of 94 up-regulated genes identified in the array, 25 were found in human PCa patients' data base and 14 of them presented one or more down-regulated miRNAs associated. After RT-qPCR and western blot validation, 3 genes and 5 miRNAs were well-established as potential molecules to further study their relevant role in bone metastasis. The overexpression of miR-135b and miR-19a decreased VTI1b (that had been found overexpressed in PC3-BM) both at the RNA and protein levels. Interestingly the modulation of these two miRNAs that presented low levels in the bone metastatic subclon, reduced the migratory capacity of PC3-BM in the presence of osteoblasts indicating that they could be relevant for the selection of bone as a metastatic niche.

Conclusion: Together, these data provide not only a new humanized model of bone metastasis, but also a genomic profile of molecules involved in bone metastasis, that further explored could represent new therapeutic targets to treat advanced stages of PCa.