Nanostring-based analysis of transcriptional metabolic signatures in Adipose-derived Stem Cells treated with epigenetic drugs during osteogenic differentiation

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Regenerative medicine offers a promising approach for treating bone diseases, particularly those involving large and complex defects. This approach has the potential to reconstruct or repair damaged tissues using stem cells and biomaterials. Adipose-derived mesenchymal stem cells (ASCs) are commonly used in the clinical treatment of various disorders due to their capacity for self-renewal, ability to differentiate into multiple cell types, and their anti-inflammatory and immunomodulatory properties. Understanding the molecular mechanisms underlying ASC biology is crucial for identifying key pathways that regulate differentiation, thereby allowing to enhance their intrinsic characteristics and develop more efficient regenerative strategies. In this context, metabolic processes play a crucial role in regulating stem cell functions such as proliferation, quiescence, response to cellular stressors, cell death and epigenetic control. In the present study, we aimed to thoroughly investigate the molecular pathways involved in the osteogenic differentiation of ASCs, with a particular focus on cellular metabolism. We employed Nanostring nCounter, an advanced digital sensing method capable of directly profiling single molecules in a single reaction without the need for enzymatic reactions, for highly multiplexed analysis. This technology enabled comprehensive characterization of differentially regulated transcripts in undifferentiated versus osteogenically differentiated ASCs, as well as in ASCs treated with RG-108, a pan-DNMT inhibitor, during a 21-day differentiation period. Subsequent *in silico* annotation and gene ontology analysis identified specific genes and pathways that are dynamically modulated during ASC-driven osteogenic differentiation. In RG-108-treated cells, we observed statistically significant alterations in osteogenic differentiation-relevant genes, such as the well-known marker RUNX2 and the new candidates AOX1 and ADH1A/1B/1C, which encode for key enzymes associated with a variety of osteoblast-related metabolic processes. Additionally, we are evaluating the differentiation potential of ASCs under dynamic conditions by using the IVTech fluidic platform, which recreates a more physiological and realistic cell culture environment, providing insights into differences compared to standard static cell cultures. Our findings will be useful to shed light on the effects of epigenetic drugs in enhancing ASC properties for the development of effective and personalized clinical solutions.

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