196 USE OF A MICRO-BIOREACTOR TO PROMOTE 3-DIMENSIONAL CELL REARRANGEMENT AND INDUCE, MAINTAIN, AND STABILIZE HIGH PLASTICITY IN EPIGENETICALLY ERASED FIBROBLASTS

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Abstract

Development and cell differentiation are driven by complex epigenetic mechanisms that regulate chromatin structure and specific gene transcription programs. We recently demonstrated that it is possible to modify the epigenetic signature of terminally differentiated cells, switching their phenotype into one of higher plasticity, through the use of molecules that remove epigenetic marks from DNA and histones (Pennarossa *et al.* 2013 Proc. Natl. Acad. Sci. **110**, 8948–8953; Brevini *et al.* 2014 Stem Cell Rev. **10**, 633–642). Here we drive mammalian fibroblasts into a high plasticity state using the epigenetic eraser, 5-aza-cytidine (5-aza-CR), and investigate whether the simultaneous use of a micro-bioreactor culture system is able to promote three-dimensional (3D) cell rearrangement, boost the induction of high plasticity, and stably maintain it. To this purpose, fibroblasts were either plated on plastic dishes (Group A) or encapsulated in a liquid marble micro-bioreactor (polytetrafluoroethylene powder; Sigma 430935, St. Louis, MO; Group B). Both groups were erased with 5-aza-CR and cultured in embryonic stem cell medium for 28 days. Morphological analysis was carried out for the entire length of the experiment. The *OCT4*, *NANOG*, and *REX1* expression levels were assessed by real-time PCR at different time points. Exposure to 5-aza-CR induced a dramatic change in morphology in Group A fibroblasts. Cells became rounded, with larger and granulated nuclei and retained a monolayer distribution for the entire length of the experiment. The same changes in cell and nuclear morphology were observed also in cells encapsulated in liquid marble (Group B). In addition, these cells formed 3D spherical structures that were stably maintained until Day 28. These morphological rearrangements were accompanied by the active expression of the pluripotency markers, *OCT4*, *NANOG*, and *REX1*, in both groups. However, while Group A cells progressively down-regulated their expression by Day 6, Group B cells steadily transcribed these genes until Day 28, when cultures were arrested. Altogether, the data confirm that epigenetic erasing induces a high plasticity state in terminally differentiated fibroblasts with the expression of pluripotency related genes. Striking morphological changes accompanied the removal of epigenetic marks. These were influenced by the use of an adequate 3D *in vitro* culture system, with the induction of distinctive cell rearrangements and the formation of spherical structures that boosted and maintained cell plasticity. These results suggest a correlation between the mechanotransduction pathways induced by the micro-bioreactor culture system and the epigenetic regulation of cell phenotype.
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