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LIVE TRACKING OF HETEROCHROMATIN REMODELLING IN MESENCHYMAL STEM CELLS DURING ADIPOGENIC AND OSTEOGENIC DIFFERENTIATION

T. X. Wu[1], K. Y. Kulebyakin[2], L. V. Putlyaeva[2,3]

[1]*Department of Biochemistry, Faculty of Biology, Lomonosov Moscow State University*
[2]*Laboratory of Molecular Endocrinology, Institute of Regenerative Medicine, Medical Research and Educational Institute, Lomonosov Moscow State University*
[3]*Department of Biochemistry and Regenerative Biomedicine, Faculty of Fundamental Medicine, Lomonosov Moscow State University*

✉ sianbaovitch@gmail.com

Abstract

Using epigenetic fluorescent sensors, we have shown that heterochromatin remodeling unfolds in opposing directions for adipogenic and osteogenic differentiation. Furthermore, addition of 100 μM indomethacin to adipogenic differentiation did not strongly affect dynamic of heterochromatin remodeling despite inducing rapid formation of lipid droplets.

An MSC ASC52telo cell line expressing an epigenetic sensor was created using lentiviral transfection and FACS. This sensor consists of two MPP8 CHROMO-domains, mTurquoise2 and NLS [1]. The cells were differentiated into adipogenic and osteogenic lineages and heterochromatin patterns of nuclei were registered in live cells. Images of single nuclei were analyzed using 98 image features (Haralick features, TAS features, Zernike moments and chromatin features) to describe epigenetic patterns. These feature values were then averaged and used for clustering by employing PCA for dimensionality reduction and data projection into a 2D graph (fig. 1).

Machine learning analysis of H3K9me3 rearrangement during differentiation detected early changes in heterochromatin organization as early as day 2, which continued to change throughout differentiation. Heterochromatin patterns of adipogenic and osteogenic differentiation diverged at early stages and did not converge at any point in time (fig. 2, *b*). Comparison of adipogenic differentiation with and without addition of 100 μM indomethacin, an allosteric PPAR-γ ac-

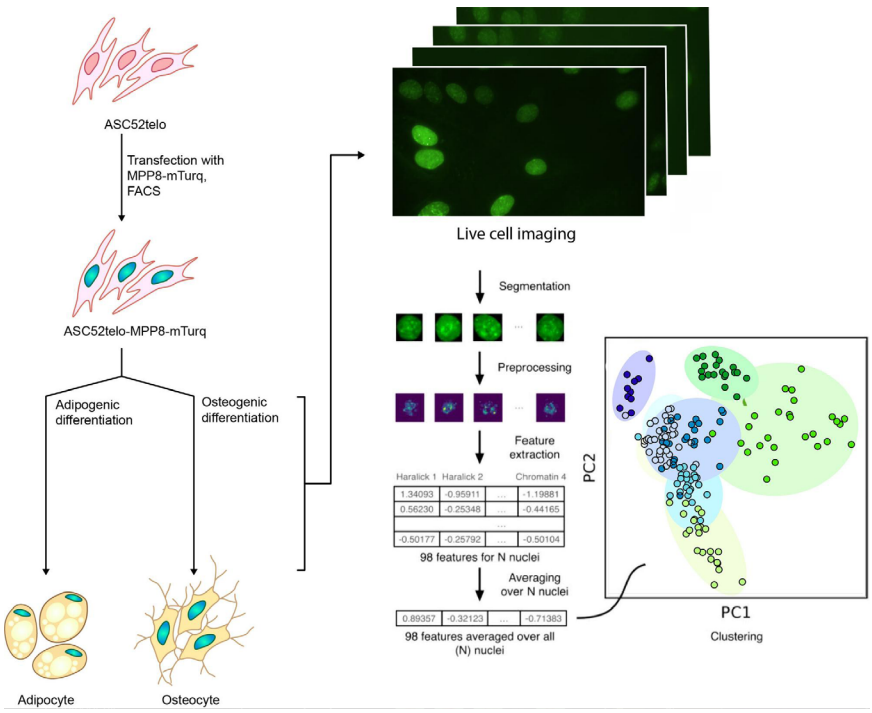

Fig. 1. Analysis of epigenetic remodeling

tivator, showed that despite higher lipid droplet formation in samples with indomethacin (fig. 3), the overall dynamics of heterochromatin remodeling remained the same (see fig. 2, *a*).

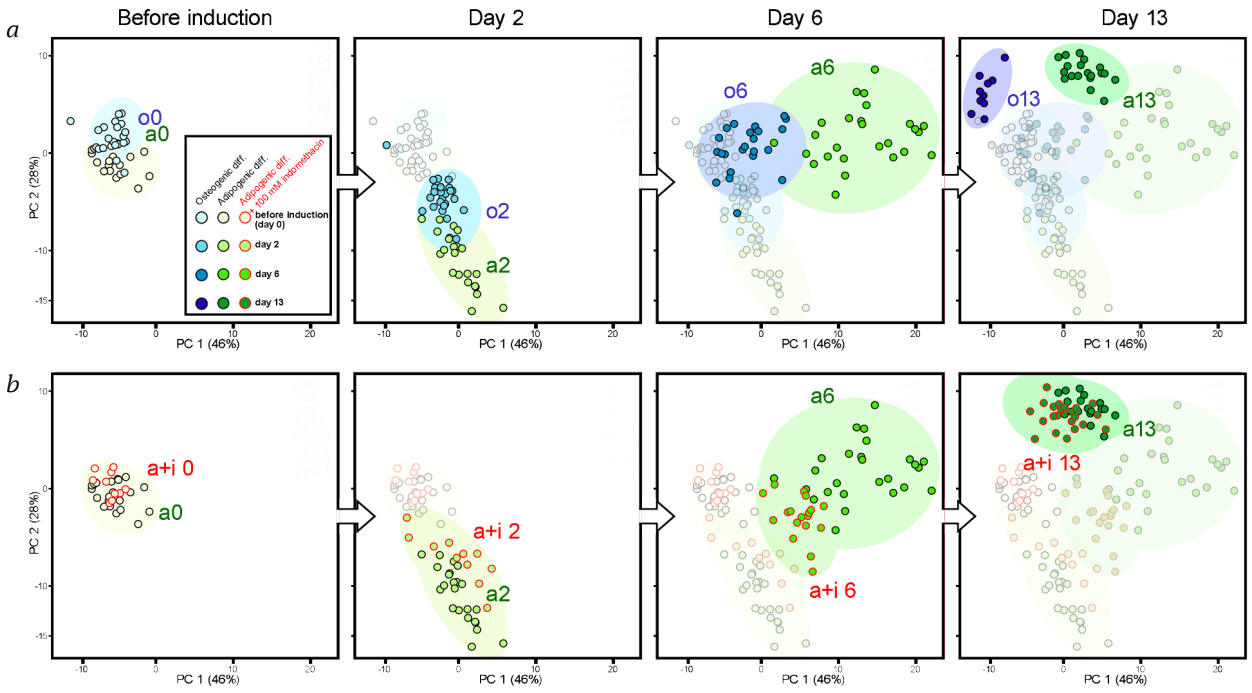

Fig. 2. Principal Component Analysis (PCA) of heterochromatin features throughout differentiation. Comparison of (*a*) adipogenic and osteogenic differentiation and (*b*) adipogenic with and without 100 μM indomethacin

Our research highlights the potential of epigenetic sensors as a sensitive tool for tracking cell processes at single cell level. The next step in our work is to further analyze epigenetic remodeling in other key processes, such as dedifferentiation, aging and oncogenesis [2, 3].

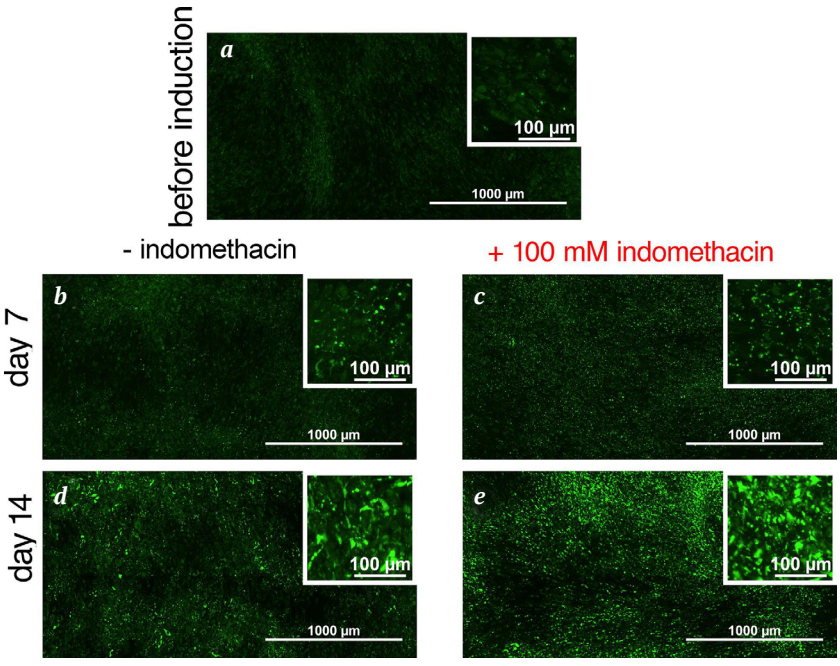

Fig. 3. Nile Red staining of lipid droplets in adipogenic differentiation (*a*) before differentiation induction (*b*, *c*) 7 days and (*d*, *e*) 14 days after induction

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