Biophysical Cues Regulate Nanoscale Chromatin Organization in Mesenchymal Stem Cells

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Introduction

Mesenchymal stem cells (MSCs), a promising cell source for musculoskeletal therapies, are subjected to mechanical forces during regeneration and repair.[1,2] Exogenous biophysical cues, such as changing substrate stiffness (SS) and fluid-induced shear stress (FSS), can direct lineage specification via chromatin reorganization.[2,3] We recently showed that dynamic tensile loading or FSS caused rapid chromatin reorganization (within 30 min) in MSC nuclei, mediated by histone methylation (e.g. H3k27me3).[3,4] These changes in chromatin organization resulted in changes in gene expression and cell differentiation.[5] However, since the length scale of chromatin complexes in the nucleus is very small (< 100 nm), obtaining well-resolved images of their interaction using conventional microscopy remains challenging. Here, we assessed how biophysical cues (SS or FSS) altered the organization of histone-H2B (H2B) at the nanoscale in MSCs, as well as the spatial localization of specific histone modifications, using a super-resolution nanoscopy [i.e. stochastic optical reconstruction microscopy (STORM)].

Methods

Human MSCs were isolated from unprocessed human bone marrow (male, 22 years, LONZA). To investigate the effect of SS, MSCs (passage #1; 3,000 cells/cm^2) were cultured on 2 well-chambered cover glass (Glass;Stiff) or 5 kPa (soft) methacrylated hyaluronic acid hydrogels (MeHA) modified with RGD to promote cell adhesion.[6] followed by 2 days of culture in basal cell growth media. To investigate the role of the histone H3k27 methyltransferase EZH2 in chromatin reorganization, cells were treated with GSK343 (GSK, 2.5μM, Sigma) for 1 day before imaging. To investigate the effects of FSS, a custom-PDMS microfluidic chamber was developed[4] to impose FSS to MSCs seeded on a chambered cover glass (500 cells/mm^2) at different shear stress conditions (1 or 5 dyne/cm^2) and for different durations (30 min or 2 hour). After the cessation of loading, cells were immunostained for histone-H2B (H2B, Proteintech) or H3k27me3 (a mark for condensed chromatin and transcriptional inactivation, Abcam), and then incubated with secondary antibodies custom-labeled with activator-reporter dye pairs (Alexa Fluor 405-Alexa Fluor 647, Invitrogen) for STORM imaging (Nanoimager, ONI).[7] STORM images were analyzed and rendered using Nanoimager software (ONI). For quantitative analysis, Voronoi tessellation of the H2B localizations was adapted to segment super-resolution images in MATLAB[7], and heat maps based on the density of H2B localizations were generated.[8]

Results

Super-resolution images of Histone-H2B in MSC nuclei were successfully obtained, revealing the histone nanodomains, which could not be observed with conventional microscopy (Conventional, Fig. 1A). STORM images and Voronoi tessellation analysis showed that, while H2B localizations clustered to form discreet and spatially separated nanodomains in MSC nuclei on glass (Fig. 1), nuclei on soft 5kPa MeHA substrates contained smaller domains (Fig. 1 A-C). Interestingly, heat maps showing the density of H2B or H3k27me3 localizations revealed that these marks were primarily localized at nuclear periphery on soft substrates (Fig. 1D, E) with no change in the total number of H2Bs per nuclear area (not shown). However, when GSK, an inhibitor of the histone H3k27 methyltransferase EZH2, was added to MSCs seeded on soft MeHA hydrogels, the distribution and localization of H2B throughout the MSC nuclei increased (Fig. 1 F, G). Similarly, STORM imaging revealed that FSS caused marked histone H2B reorganization in MSCs (Fig. 2), depending on the magnitude or duration of FSS (Fig. 2). Voronoi cluster analysis showed that FSS increased the number of localizations per histone cluster and the cluster size (Fig. 2). FSS also increased repressive histone modifying marks (i.e., H3k27me3 localizations) in these nuclei (not shown).

Discussion

In this study, we investigated the role of biophysical cues on nanoscale chromatin reorganization in MSC nuclei using STORM imaging. Our findings showed that substrate stiffness regulates chromatin organization at the nanoscale, with stiff substrates resulting in H2B and H3k27me3 nanodomains that

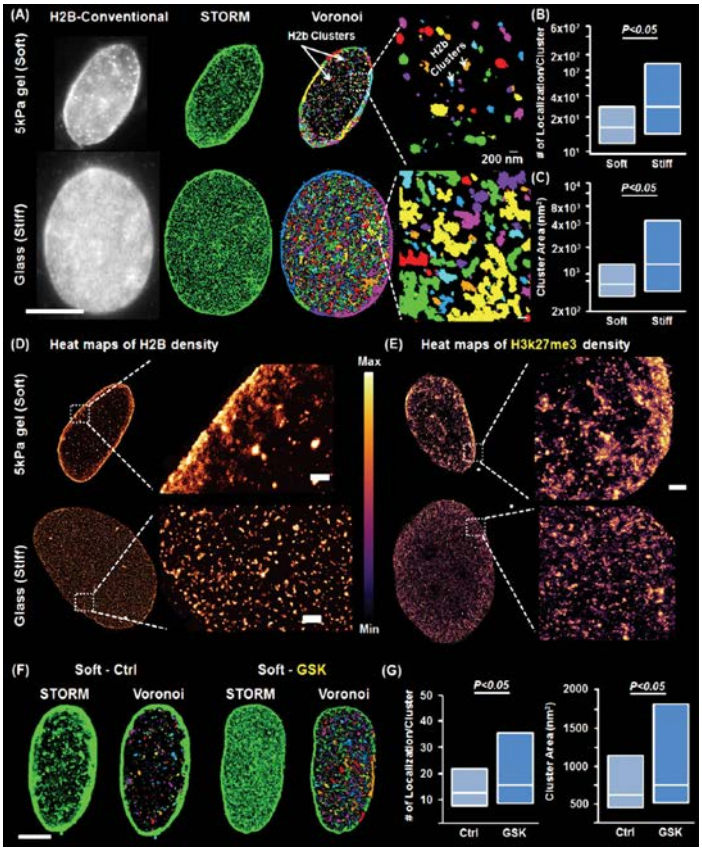

Figure 1. (A) Conventional image, STORM image, and Voronoi cluster analysis of H2B localizations in MSC nuclei on soft and stiff substrates, scale bar = 5 μm; (B and C) Quantification of the number of H2B localizations per cluster and the cluster area (n ≥ 12,345 clusters from 5 cells); (D, E) heat maps showing H2B or H3k27me3 localization density on soft and stiff substrates, scale bar = 500 nm. (F, G) STORM image, Voronoi cluster analysis, and quantifications of H2B localizations for cells on soft substrates with/without GSK treatment for 1 day (scale bar = 5 μm, n ≥ 11,227 clusters from 5 cells). The box and line correspond to the interdecile range (IDR, 10th~90th percentile) and median, respectively.

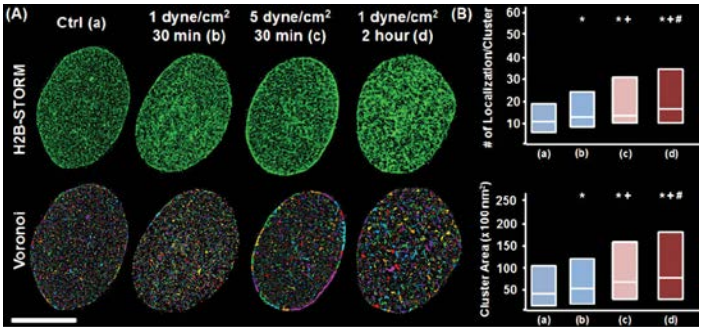

Figure 2. (A) STORM image and Voronoi cluster analysis of H2B localizations in MSC nuclei in response to FSS, scale bar = 5 μm; (B) Quantification of the number of H2B localizations per cluster and cluster area (n ≥ 10,545 clusters from 5 cells. The box and line correspond to the interdecile range (IDR, 10th~90th percentile) and median, respectively (*p<0.05 vs. a, +p<0.05 vs. b, #p<0.05 vs. c).

were distributed throughout the nuclei. Conversely, on soft substrates, these domains shifted to the nuclear periphery, a region in which heterochromatin dominates and methyltransferases are abundant.[9] When MSCs on these soft substrates were treated with GSK, an inhibitor of histone H3k27 methyltransferase EZH2, the localization to the nuclear periphery was prevented. This suggests that physical forces, acting through EZH2, may play an important role in nanodomain translocation to the nuclear periphery. We also noted that other physical forces, such as FSS, caused rapid histone condensation and reorganization, as well as increases in H3k27me3. Taken together, this study suggests that biophysical perturbations regulate nanoscale chromatin spatial organization through histone methylation in MSCs. Ongoing studies are focused on elucidating how these histone reorganizations and modifications by biophysical cues act to regulate gene expression and enforce lineage specification in MSCs.

Significance

Here, we show that biophysical cues regulate chromatin organization at the nano-scale using super-resolution imaging. These studies may help to elucidate epigenetic mechanisms regulating stem cell differentiation and enhance their use in regenerative medicine applications.

Acknowledgements

This work was supported by the National Institutes of Health (R01 AR056624).

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