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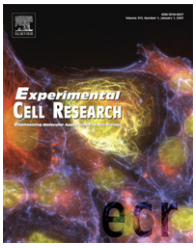

Research Article

Cell shape regulates global histone acetylation in human mammary epithelial cells

Johanne Le Beyec[b,c,d,1], *Ren Xu*[a,1], *Sun-Young Lee*[a], *Celeste M. Nelson*[a], *Aylin Rizki*[a], *Jordi Alcaraz*[a], *Mina J. Bissell*[a,*]

[a]*Life Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, MS 977 R225A, Berkeley, CA 94720, USA*
[b]*Centre de recherche des Cordeliers, Université Piere et Marie Curie-Paris 6, ULMRS872, Paris F-75006, France*
[c]*INSERM, UMR S 872, Paris F-75006, France*
[d]*Université Paris Descartes-Paris 5, UMR S 872, Paris F-75006, France*

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ABSTRACT

Extracellular matrix (ECM) regulates cell morphology and gene expression in vivo; these relationships are maintained in three-dimensional (3D) cultures of mammary epithelial cells. In the presence of laminin-rich ECM (lrECM), mammary epithelial cells round up and undergo global histone deacetylation, a process critical for their functional differentiation. However, it remains unclear whether lrECM-dependent cell rounding and global histone deacetylation are indeed part of a common physical–biochemical pathway. Using 3D cultures as well as nonadhesive and micropatterned substrata, here we showed that the cell 'rounding' caused by lrECM was sufficient to induce deacetylation of histones H3 and H4 in the absence of biochemical cues. Microarray and confocal analysis demonstrated that this deacetylation in 3D culture is associated with a global increase in chromatin condensation and a reduction in gene expression. Whereas cells cultured on plastic substrata formed prominent stress fibers, cells grown in 3D lrECM or on micropatterns lacked these structures. Disruption of the actin cytoskeleton with cytochalasin D phenocopied the lrECM-induced cell rounding and histone deacetylation. These results reveal a novel link between ECM-controlled cell shape and chromatin structure and suggest that this link is mediated by changes in the actin cytoskeleton.

Introduction

Cell structure and function were postulated to be intimately connected in maintenance of tissue homeostasis [1]. Manipulating cellular and nuclear morphology in culture can induce a variety of functional changes, including glucose uptake and metabolism [2], proliferation [3–5], apoptosis [4], differentiation and gene expression [6–10]. These morphologically driven processes are controlled by the local microenvironment as exemplified in the mammary gland and mammary epithelial cells (for a review see [11]). Isolated mouse mammary epithelial cells can be induced to undergo structural and functional

* *Corresponding author*. Fax: +1 510 4865586.
E-mail address: mjbissell@lbl.gov (M.J. Bissell).

Abbreviations: ECM, extracellular matrix; lrECM, laminin-rich reconstituted ECM; HATs, histone acetyltransferases; HDACs, histone deacetylases; polyHEMA, poly(2-hydroxyethyl methacrylate); TPA, 12-o-tetradecanoylphorbol 13-acetate; 2D, two-dimension(al); 3D, three-dimension(al); AcH3 and AcH4, acetylated histone H3 and H4; ChIP, chromatin immunoprecipitation

[1] J.L.B. and R.X. contributed equally to this work.

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differentiation in three-dimensional (3D) cultures. The acquisition of the functionally differentiated phenotype, i.e. expression of milk proteins, requires lactogenic hormones and the basement membrane protein laminin-111 (laminin-1) [12,13]. The latter induces dramatic morphological changes in that cells become rounded and actin becomes cortical. These morphological changes are necessary for expression of some milk proteins including β-casein [9]. Artificially pre-rounding the cells by plating on the nonadhesive substratum poly(2-hydroxyethyl methacrylate) (polyHEMA) poises them for responsiveness to prolactin and laminin-rich ECM (lrECM)[9], whereas preventing cell rounding by treatment with the phorbol ester 12-o-tetradecanoylphorbol 13-acetate (TPA) inhibits milk protein synthesis [14]. These previous findings strongly suggested that cell rounding, mediated by reorganization of actin filaments and other cytoskeletal components, may be an important physical signal conveyed by the ECM leading to changes in gene expression.

Cell fate and differentiated function are controlled by patterns of gene expression, which in turn are dictated by chromatin organization. The structure of chromatin is regulated by a number of post-translational modifications at the amino-terminal tails of nucleosomal histones, including acetylation/deacetylation, phosphorylation, methylation, and ADP ribosylation [15]. The acetylation status of chromatin is dynamic, balanced by the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) [16]. Gene expression generally correlates with histone hyperacetylation at promoter regions and other regulatory *cis*-elements [17–19] whereas histone deacetylation represses transcription by promoting chromatin condensation into heterochromatin [20,21]. Histone deacetylation was shown to correlate with changes in gene expression in the developing brain in rats [22], and abrogating the deacetylation by treatment with an HDAC inhibitor resulted in impaired brain development and delayed expression of differentiation markers. Functional differentiation of many cell types is associated with activation of specific subsets of genes, silencing of other genes, and extensive formation of heterochromatin [23].

Acinar morphogenesis of a non-malignant human mammary epithelial cell line HMT3522-S1 is accompanied by rearrangements in chromatin structure and nuclear architecture [24–26]. These reorganizations appear to be important for establishing and maintaining the normal phenotype of cells in 3D [24,25,27]. We previously found that the global level of histone acetylation is critical for functional differentiation of mammary epithelial cells [24,28]. Altering chromatin structure by increasing histone acetylation with trichostatin A induces human mammary epithelial cells to alter their morphology, re-enter the cell cycle, and become disorganized [24]; overexpressing an HAT in mouse mammary epithelial cells inhibits production of endogenous β-casein in response to lrECM [28]. Conversely, treatment with lrECM induces global histone deacetylation in human and mouse mammary epithelial cells [25,28].

These previous studies showed that ECM has profound effects on cell shape and chromatin structure, particularly histone deacetylation, but it was not understood whether these effects were linked. In this report, we used several strategies to change cell shape, including 3D culture on different substrata with different adhesiveness and micropatterned substrata. We find that cell rounding per se induces global histone deacetylation and an increase in chromatin condensation and that these processes are associated with a global reduction of gene expression. These results reveal a process by which ECM integrates structure and function in mammary epithelial cells through a shape-dependent global histone deacetylation.

Materials and methods

Cell culture

HMT-3522-S1 and -T4-2 human mammary epithelial cells [29,30] were grown as two-dimensional (2D) monolayers on plastic or within 3D lrECM (Matrigel, Collaborative Research) and maintained as previously described [31,32]. Cells were grown in 2D and 3D for 10 days before harvests. For culture on nonadhesive substrata, ~4000 cells/cm^2 were seeded on polyHEMA (Sigma)-coated plates prepared as previously described for mouse mammary epithelial cells [9].

Micropatterning

Micropatterned substrata consisting of collagen-coated islands were created as described [33]. Briefly, elastomeric stamps containing a relief of the desired pattern were coated with type I collagen (50 μg/mL in water; Vitrogen 100, Cohesion Technologies, Palo Alto, CA) for 2 h, washed with water, and dried under a stream of nitrogen. Flat poly(dimethylsiloxane) (PDMS; Sylgard 184, Ellsworth Adhesives, Germantown, WI) elastomer-coated substrata were UV-oxidized for 7 min (UVO Cleaner, Jelight Co., Irvine, CA), stamped with collagen, blocked with 1% pluronic F108 (BASF Corp., Florham Park, NJ) in water for 1 h, and rinsed in PBS before seeding T4-2 cells. Cells were allowed to attach to the patterned islands for approximately 30 min before washing away the remaining floating cells. A flat block of PDMS was coated with collagen and stamped for the unpatterned substratum control. For measurements of projected cell area, phase contrast images of individual cells were outlined and processed with Scion Image software.

Global gene expression analysis

cDNA microarrays with ~8000 known genes spotted on poly-L-lysine-coated chips (custom arrayed at Lawrence Berkeley National Laboratory using Research Genetics 8k human clones) were used. mRNA samples of interest were directly compared to each other by co-hybridization to the same slide using dendrimer technology to label with red-Cy5 and green-Cy3 (Genisphere). Total RNA (1 μg) isolated with Qiagen RNEasy reagents was used for each sample hybridized. Cells in 3D lrECM were extracted using 5 mM EDTA in cold PBS to dissolve the Matrigel. For each comparison, 3 independent sets of cells cultured for 10 days were processed, and 4 slides were hybridized. This corresponded to 3 sets of RNA from independent culture sets plus a dye-swap experiment in which the red and green label was switched for the two samples in question to account for dye-specific effects. Arrays were scanned using a Genepix scanner (Axon). Raw data for each channel (red and green) were loaded onto Genespring (Silicon Genetics) for normalization and analysis. For each chip, per-spot and per-chip intensity-dependent Lowess