Tissue phenotype depends on reciprocal interactions between the extracellular matrix and the structural organization of the nucleus

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**ABSTRACT** What determines the nuclear organization within a cell and whether this organization itself can impose cellular function within a tissue remains unknown. To explore the relationship between nuclear organization and tissue architecture and function, we used a model of human mammary epithelial cell acinar morphogenesis. When cultured within a reconstituted basement membrane (rBM), HMT-3522 cells form polarized and growth-arrested tissue-like acini with a central lumen and deposit an endogenous BM. We show that rBM-induced morphogenesis is accompanied by recanalization of the nuclear matrix proteins NuMA, splicing factor SRm160, and cell cycle regulator Rb. These proteins had distinct distribution patterns specific for proliferation, growth arrest, and acini formation, whereas the distribution of the nuclear lamina protein, lamin B, remained unchanged. NuMA localized to foci, which coalesced into larger assemblages as morphogenesis progressed. Perturbation of histone acetylation in the acini by trichostatin A treatment altered chromatin structure, disrupted NuMA foci, and induced cell proliferation. Moreover, treatment of transiently permeabilized acini with a NuMA antibody led to the disruption of NuMA foci, alteration of histone acetylation, activation of metalloproteases, and breakdown of the endogenous BM. These results experimentally demonstrate a dynamic interaction between the extracellular matrix, nuclear organization, and tissue phenotype. They further show that rather than passively reflecting changes in gene expression, nuclear organization itself can modulate the cellular and tissue phenotype.

The cell nucleus is organized by a nonchromatin internal structure referred to as the nuclear matrix (NM; refs. 1–3). Identified NM components include coiled-coil proteins (4), cell cycle regulators (5), tissue-specific transcription factors (6, 7), and RNA splicing factors (for review see ref. 2). Although splicing factors have been shown to redistribute during cellular differentiation (8, 9) and following the induction of gene expression (10), spatial distribution of nuclear components are thought to be the consequence of changes in gene expression (8, 10, 11). However, whether NM composition and structure may themselves affect gene expression and cellular function has not been examined.

To systematically study the effect of cell growth and tissue differentiation on nuclear organization, we used a reconstituted basement membrane (rBM)-directed model of mammary gland morphogenesis (12). The HMT-3522 human mammary epithelial cells (HMECs) were isolated from reduction mammoplasty and became immortalized in culture (13). When embedded within a rBM, these cells arrest growth, organize an endogenous BM, and form polarized acinus-like structures with vectorial secretion of sialomucin into a central lumen (12). We used this model to compare the nuclear organization of HMECs cultured on a plastic surface [two-dimensional (2D) monolayer] vs. a three-dimensional (3D) rBM. Nuclear organization was assessed by examining the distribution of the folded NM proteins lamin B (14) and NuMA (15), the cell cycle regulator Rb (p110Rb; ref. 5), and the splicing factor SRm160 (formerly known as B1C8; ref. 16). These proteins had distinct spatial distribution patterns specific for proliferation, growth arrest, and acini formation. Moreover, disruption of nuclear organization in acini by either perturbing histone acetylation or directly modifying the distribution of NM proteins altered the acinar phenotype.

We previously hypothesized (17) and thereafter provided evidence that the extracellular matrix (ECM) directs morphogenesis and gene expression in mammary epithelial cells (12, 18, 19). Here we show that a reciprocal relationship exists between the ECM and nuclear organization. These findings underscore a role for nuclear organization in regulation of gene expression and provide a possible framework for how cell–ECM interactions determine cell and tissue phenotype.

**MATERIALS AND METHODS**

**Cell Culture.** HMT-3522 HMECs (S-1 passage-50 cells; ref. 13) were propagated in 2D cultures in chemically defined medium (12), and growth arrest was induced by removing epidermal growth factor (EGF) for 48 hr. Cultures were prepared by embedding single cells (8.5 × 10⁵ cells per ml of matrix) in rBM (Matrigel, Collaborative Research) or collagen-I matrix (Collagen AC-5, ICN) in 4-well chamber slides (Nalge). These cultures were grown for 5–10 days. Growth arrest and morphogenesis were routinely observed by days 7–9.

**Antibodies and Inhibitors.** For Western blots and/or immunostaining, we used mAbs against type IV collagen (clone AB9, Dako), β-catenin (clone 14, Transduction Laboratories, Lexington, KY), SRm160 splicing factor (clone B1C8, 16), lamin B (clone 101-B7, Matritech, Cambridge, MA), NuMA (clone 204–41, Matritech, and clone B1C11, a gift from S. Penman, Massachusetts Institute of Technology, Cambridge, MA), and polyclonal antibodies (pAbs) against Ki-67 (NovoCastra, Newcastle, U.K.), acetylated histone H4 (Upstate Biotechnology, Lake Placid, NY), and p110Rb (Santa Cruz Biotechnology). For bioperturbation assays, we used mAbs against lamins A/C (clone 636, Novocastra, Newcastle, U.K.)

Abbreviations: NM, nuclear matrix; BM, basement membrane; rBM, reconstituted BM; HMEC, human mammary epithelial cells; 2D and 3D, two and three dimensional; Rb, retinoblastoma protein; ECM, extracellular matrix; EGF, epidermal growth factor.

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and NuMA (clone 22, Transduction Laboratories, Lexington, KY), in addition to B1C11 and 101-B7. Trichostatin A (Wako Chemicals, Richmond, VA) was used as an inhibitor of histone deacetylase (40 nM).

Indirect Immunofluorescence. Cells were permeabilized in situ (0.5% Triton X-100 in 100 mM NaCl/300 mM sucrose/10 mM Pipes, pH 7.2/7.8 mM MgCl2 containing 1 mM Pefabloc Sc (AEBSF) (Boehringer Manheim)/10 µg/ml leupeptin/10 µg/ml aprotinin/10 µg/ml trypsin inhibitor type II/250 µM NaF), fixed in 2% paraformaldehyde, and immunostained as described (18). Human mammary tissue was snap-frozen in n-hexane and embedded in Tissue-Tek O.C.T. compound (Sakura Firetek, Torrance, CA); 5-µm sections were fixed in methanol and immunostained in accordance with human protocol (KF) 01–216/93 in the laboratory of O.W.P.

Image Acquisition, Processing, and Data Analysis. Samples were analyzed by using a Bio-Rad MRC 1024 laser scanning confocal microscope attached to a Nikon Diaphot 200 microscope. Fluorescence specificity was verified by sequential fluorophore excitation and analysis for each fluorophore. Image space-3D analysis program (Molecular Probes) and normalized to 3D rBM cluster-cell number by highlighting and counting each nucleus using Image Space-Measure 2D. The voxel threshold was set at 0.2 µm.

Immunoblot Analysis. Total cell extracts (2% SDS in phosphate-buffered saline, pH 7.4, containing 1 mM Pefabloc/10 µg/ml leupeptin/10 µg/ml aprotinin/10 µg/ml trypsin inhibitor type II/250 µM NaF) were prepared in situ for 2D cultures or from acini isolated from 3D cultures by dispase treatment (5,000 units per ml caseinolytic activity, Collaborative Research). Equal amounts of protein were separated and immunoblotted as described (18).

In Situ NM Preparation. In situ NM preparation was as previously described (20), except that 0.05% Triton X-100 and micrococcal nuclease (5 units per ml) for 48 hr, after which the cells were incubated with fresh medium for an additional 48 hr. Antibody concentrations and incubation times were determined empirically. Trypan blue dye-exclusion tests and apoptosis studies verified the absence of digitonin toxicity.

RESULTS

Internal Nuclear Organization Is Remodeled When HMECs Are Cultured Within a Basement Membrane. HMT-3522 HMECs, like primary HMECs, undergo morphogenesis to form tissue-like acini when cultured in a 3D rBM (12, 18). Neither cell type undergoes acinar differentiation when cultured as 2D monolayers. In proliferating 2D cultures, NuMA was diffusely distributed in the nuclei of cells grown as monolayers (a), but reorganized into large nuclear foci in cells induced to undergo morphogenesis (acini formation) in response to a rBM (c). NuMA was distributed as multiple nuclear speckles in cells cultured as monolayer (c), whereas it was concentrated into fewer and larger speckles in the acini (f). Lamin B, in contrast, consistently localized to the nuclear periphery and within intranuclear patches (a and d). The distribution of lamin B (g), NuMA (h), and SRm160 (i) after in situ NM preparation of cells cultured in 3D rBM was similar to that observed in intact cells (d–f). Arrows indicate nuclei found within the plane of the section. (Bar = 10 µm.)

We next examined NuMA and SRm160 distribution at different stages of 3D rBM-induced morphogenesis. After embedment in rBM, cells proliferated to form small clusters by days 3–5 but lacked β-catenin at cell–cell junctions, and collagen IV staining was discontinuous (Fig. 2, a–e). After growth arrest (days 6–10), cells assembled a continuous endogenous BM and formed polarized acinus-like structures with organized adherens junctions (Fig. 2, Ad–Af). NuMA was uniformly distributed in the nuclei of proliferating cells (Fig. 2, Ba), but became concentrated into distinct foci of differing sizes after growth arrest (day 7; Fig. 2, Bb), and into larger and fewer foci on completion of morphogenesis (day 10, Fig. 2, Bc). NuMA and the splicing factor SRm160 were not colocalized in proliferating cells (Fig. 2, Bb’ and Ba’), but NuMA foci and SRm160 speckles were closer together after growth arrest (Fig. 2, Bb’ and Bb”). These spatial changes in NuMA arrangement occurred without significant modifications in the level of NuMA expression or molecular weight, as determined by using Western blot analysis (Fig. 2, Be). These experiments demonstrate that specific NM proteins undergo spatial rearrangement during rBM-induced acinar morphogenesis. Because the existence of NuMA in differentiated tissue has been questioned (21), we studied NuMA in the normal resting human mammary gland. Intense staining was observed in the epithelial cells of acini and ducts, where NuMA was distributed in foci of different sizes and resembled the acinar stages recapitulated in 3D rBM cultures (Fig. 2, Bd).

Growth Arrest Is Associated With Changes in NuMA and Rb Distribution. ECM-directed growth arrest is an early and critical step in mammary epithelial cell morphogenesis (12). To distinguish between the effect of ECM-directed growth arrest...
and changes caused by tissue structure and polarity, the localization of NuMA and SRm160 was compared between growth-arrested and proliferating cells cultured in monolayers. Less than 5% of the cells remained in the cell cycle after...
growth arrest induced by EGF removal, as indicated by the rearrangement of acetylated histone H4 distribution (Fig. 4 f vs. d). More dramatically, disruption of NuMA organization altered the acinar phenotype, as indicated by loss of the endogenously deposited BM (Fig. 4 j). Because the loss could be prevented by treatment with GM6001, a potent metalloprotease inhibitor (Fig. 4 n; ref. 26), we conclude that NuMA disruption led to induction and/or activation of a metalloprotease. Similar treatment of the acini with mAbs against lamin A/C or lamin B did not induce any change in histone H4 acetylation, BM integrity, or lamin distribution, even though these Abs reached their nuclear targets, as shown by secondary Ab staining (Fig. 4n and data not shown).

**DISCUSSION**

By modifying the cellular microenvironment, we have demonstrated that nuclear organization rearranges dramatically in HMECs after growth arrest and tissue-like acinar morphogenesis (Scheme 1). The use of the 3D-rBM culture assay has enabled us also to show that alterations of nuclear organization can modify the cellular and tissue phenotype.

Previously documented changes in nuclear organization have been broadly descriptive. By systematically analyzing the distribution of three NM proteins in 2D and 3D cultures, we have determined that precise nuclear rearrangements occur
with growth arrest and after rBM-induced morphogenesis. In 3D rBM cultures, both NuMA and Rb were diffusely distributed in the nucleus of proliferating cells. After growth arrest, NuMA was relocalized into discrete foci, whereas Rb redistributed into a central nuclear mass. These patterns of distribution were different from those observed in growth-arrested cells in monolayer 2D cultures, suggesting that there may be different states of growth arrest in 2D and 3D rBM cultures (27). Because NuMA distribution in 3D collagen I cultures was comparable to that observed in growth-arrested 2D cultures, our results suggest that 3D organization of cells per se cannot explain the differences seen between monolayer and 3D rBM
Dynamics of the distribution of NM proteins in 3D rBM

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