COMMENTARY

# Squeezing cells through the epigenetic machinery

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In interstitial tissues, cells are present in physically confined spaces created by the extracellular matrix (ECM). A deeper understanding of how cells disperse and migrate in confinement has important clinical implications in designing cellular therapeutics for optimal delivery to target tissues and developing treatment strategies against cancer metastasis. The mesh size of the ECM is often smaller than the cell size and hence acts as a physical barrier for cell migration. While cells produce metalloproteinases (MMPs) to degrade the ECM as they migrate (1), some cells can also migrate by undergoing physical deformation and squeezing through the ECM. The ability of cells to deform is inherently linked to their mechanical properties. For example, a classic micropipetting study shows that immature myeloid progenitors residing in bone marrow are more rigid than circulating mature blood cells (2). At the cell surface (cortex), the actomyosin cytoskeleton generates contractile forces to generate tension, which in turn regulates cell deformation (3). While the role of actomyosin contractility in migration has been widely investigated (4), the nucleus is the largest organelle in cells, and changes in nuclear morphology during cell migration were already observed more than a century ago (5). Recent studies show that lamins, the intermediate filament proteins delineating the inner nuclear membrane, regulate mechanical properties of the nucleus (6) and limit migration through small pores (7–9). Lamins physically interact with chromatin and regulate histone methylation (10). Thus, we are only beginning to understand how different components of the nucleus, such as the epigenetic machinery, impact cell migration in confined spaces. In PNAS, Wang et al. (11) report a significant step in this direction by showing that 3D environments activate actomyosin contractility to induce trimethylation of the histone 3 protein at the residue K4 (H3K4me3) via the WD repeat domain 5 (WDR5), which is required for nuclear softening and confined migration of cells (Fig. 1).

Both cell-intrinsic and -extrinsic biophysical cues influence confined migration. Blood and immune cell

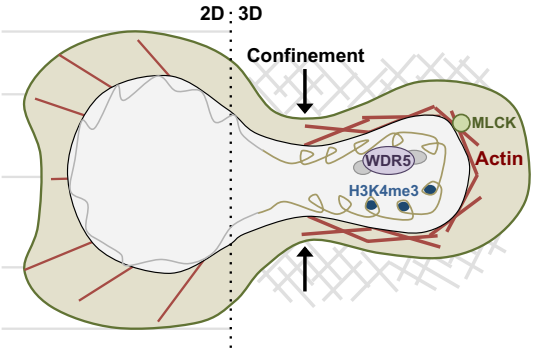


**Fig. 1. Confining cells in 3D collagen facilitates migration by increasing nuclear compliance through H3K4 methylation. Embedding cells in 3D collagen increases actomyosin contractility via MLCK, which subsequently activates WDR5 to induce H3K4me3. This process loosens chromatin, increases nuclear deformability, and is required for confined migration. In contrast, cells on 2D substrates or plastic culture show stiffer, more viscous nuclei than confined cells.**

lineages show the striking diversity in nuclear rigidity and lamin expression, which differentially impacts cell trafficking between marrow and circulation (8). This diversity is also observed in nonhematopoietic tissues, because lamin-A scales with tissue stiffness (12). While nuclear rigidity can be intrinsically programmed during development (13), cells generate contractile forces to sense mechanical properties of the ECM and subsequently alter the nuclear architecture. For example, increasing stiffness of the ECM induces structural changes in lamin-A to promote its polymerization by dephosphorylation, thereby increasing nuclear rigidity (14). This process may serve as a mechanism to protect the nucleus from DNA damage by limiting confined migration (7). Thus, active mechanisms are required to enable cell migration in confined spaces. Mature immune cells are highly deformable as they can migrate through pores as small as ~3 μm in size without requiring matrix degradation by MMPs (15). However, even these cells require a mechanism to repair the

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nuclear envelope because it ruptures during migration (16, 17). Still, it has remained untested whether confinement can actively promote migration by altering nuclear mechanics.

Cells in confined spaces show changes in chromatin, including up-regulation of H3K4 methylation by WDR5 (18) and increased fluidity of heterochromatin (19). Physical stretching of cells is also known to influence chromatin conformation, epigenetic signatures, and gene transcription (20, 21). However, the relevance of these insights in confined migration has not been studied. To address this issue, Wang et al. (11) perform knockdown experiments to show that WDR5 is required for migration in 3D collagen in vitro and extravasation in vivo. WDR5 is required for formation of trailing trails on VCAM1, while inhibiting transcription by actinomycin D does not impair trailing trail formation. Although the authors show that WDR5 knockdown does not impact cell surface receptors and cytoskeletal proteins, global-expression profiling will help clarify whether any transcriptional change upon down-regulation of WDR5 (22) can potentially impact migration. Consistent with a previous study in engineered microgrooves (18), Wang et al. (11) show that culturing cells in 3D but not on 2D collagen up-regulates H3K4me3 via WDR5. Bovine collagen was used, which is known to yield the pore diameter 2 to ∼6 μm when polymerized at ∼2 mg/mL, 37 °C (15), and hence will likely constrain embedded lymphocytes (∼10 μm in diameter). In addition, transglutaminase 2 (TGM2), which is known to enhance cross-linking of collagen while maintaining pore size (23), increases H3K4me3. While increased matrix cross-linking generally leads to higher stiffness, other biophysical factors that impact cell biology, such as ligand tethering (24) and stress relaxation (25), can also be influenced by TGM2. Thus, using engineered matrices with independent control of biophysical parameters can help clarify whether ECM mechanics can control epigenetic changes independently of biochemical properties of the ECM.

Chemical modification of histone impacts chromatin condensation (26), which in turn regulates mechanics of DNA and its interaction with enzymes involved in replication and transcription (27). Wang et al. (11) show that chromatin becomes less compact and more sensitive to DNA digestion when cells are in 3D collagen. As with a previous finding (28), Wang et al. (11) show that decreased DNA compaction is associated with increased compliance of the nucleus. Importantly, WDR5 is required for migration through 3 μm, but not larger pores. In contrast, lamin-A limits confined migration (7–9). While the mechanistic link between lamin-A and H3K4 methylation was not tested, lamina-associated domains are generally depleted of H3K4me3 (10). Taken together, the results suggest a possible antagonistic relationship between lamin-A and the machinery that regulates H3K4me3.

Actomyosin filaments are connected to the nuclear envelope via the linkers of nucleoskeleton and cytoskeleton complexes (29). Thus, sensing of physical cues from the ECM by generating contractile forces can, in principle, rapidly induce structural changes in the nucleus (30). Consistent with this notion, Wang et al. (11) show that up-regulation of H3K4me3 in confined cells requires actomyosin contractility by activating myosin light chain kinase (MLCK), which is subsequently recruited to the nucleus to activate WDR5. Although not directly tested in this study, the results suggest an intriguing possibility that in some cases, contractile forces could soften the nucleus in confinement to drive 3D migration. Previous studies with engineered elastic hydrogels show that increased contractility by matrix stiffening rigidifies the nucleus (12, 14), which could impair confined migration. However, collagen gels and other naturally derived matrices undergo stress relaxation as cells generate physical forces; this process further drives cellular contractility, spreading, and motility independently of initial matrix stiffness (25). Because collagen gels are as soft as marrow (15), actomyosin contractility generated by cells in 3D collagen could potentially come from stress relaxation, rather than stiffness of the ECM. Thus, it will be important to elucidate whether stress relaxation of the ECM can regulate chromatin conformation via histone methylation, while stiffness regulates nuclear lamins.

**Wang et al. show that chromatin becomes less compact and more sensitive to DNA digestion when cells are in 3D collagen.**

The idea that the epigenetic machinery can control nuclear mechanics and confined cell migration is striking. Thus, the work of Wang et al. (11) raises a number of important questions. First, the role of the epigenetic machinery and nuclear mechanics in different modes of migration needs to be clarified, because the extent of actomyosin contractility and cell-matrix adhesion determines whether cells undergo lobopodial, lamellipodial, or amoeboidal movement (31). Molecular engineering approaches (32) can be used not only to address this issue but also to perform proof-of-concept studies on whether directly changing chromatin conformation impacts migration and retention of therapeutic cells delivered in the body. In addition, it remains to be determined how up-regulation of H3K4me3 during confined migration leads to long-term changes in gene expression. For example, actin polymerization increases translocation of megakaryocytic leukemia 1 into the nucleus (33), which then recruits H3K4me3 to activate transcription of proinflammatory genes via NF-κB (34). This suggests an interesting possibility that confined migration could prime some cells, such as lymphocytes, to respond to inflammation. Ultimately, it will be important to test whether cells can "remember" a biophysical state of their surrounding ECM (35) and subsequently pass on their epigenetic information to daughter cells (36) to influence their mechanical behaviors.


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