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Past matrix stiffness primes epithelial cells and regulates their future collective migration through a mechanical memory

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ABSTRACT

During morphogenesis and cancer metastasis, grouped cells migrate through tissues of dissimilar stiffness. Although the influence of matrix stiffness on cellular mechanosensitivity and motility are well-recognized, it remains unknown whether these matrix-dependent cellular features persist after cells move to a new microenvironment. Here, we interrogate whether priming of epithelial cells by a given matrix stiffness influences their future collective migration on a different matrix – a property we refer to as the 'mechanical memory' of migratory cells. To prime cells on a defined matrix and track their collective migration onto an adjoining secondary matrix of dissimilar stiffness, we develop a modular polyacrylamide substrate through step-by-step polymerization of different PA compositions. We report that epithelial cells primed on a stiff matrix migrate faster, display higher actomyosin expression, form larger focal adhesions, and retain nuclear YAP even after arriving onto a soft secondary matrix, as compared to their control behavior on a homogeneously soft matrix. Priming on a soft ECM causes a reverse effect. The depletion of YAP dramatically reduces this memory-dependent migration. Our results present a previously unidentified regulation of mechanosensitive collective cell migration by past matrix stiffness, in which mechanical memory depends on YAP activity.

1. Introduction

Mechanical properties of the extracellular matrix (ECM) influence phenotypic and genotypic cellular responses, which can impact cell differentiation, migration, and proliferation [1–4]. In particular, matrix stiffness regulates cellular forces, adhesions, protrusions, and polarization through mechanotransductive signaling, all of which lead to mechanosensitive variations in migration phenotypes of both single and grouped cells [3,5–7]. Physiologically, migratory cells do not continually interact with just one type of matrix. Instead, grouped cells migrate through mechanically heterogeneous matrices, forming the basis of fundamental biological processes including embryonic development, wound healing, regeneration, and cancer metastasis [8]. During embryonic development, a coordinated collective movement of epithelial cells across diverse microenvironments enables branching morphogenesis, a process necessary for organ formation [9,10]. In cancer metastasis, the mechanical properties of the primary tumor microenvironment are known to induce de-clustering [2,11,12] and outward migration [5,13] of cancer cells into the mechanically dissimilar surrounding tissue, which represent the first steps of tumor invasion.

As cells pass through a tissue microenvironment, a distinct set of mechanosensitive signaling events occur [1], such as clustering of integrin-based adhesion proteins into focal adhesions [14], Rho-ROCK activation [15,16], and nuclear localization of transcriptional regulators YAP and SNAIL1 [17,18]. Recently, mechanical dosing of human mesenchymal stem cells on matrices of tunable stiffness has been found to regulate mechanical memory-dependent lineage commitment decisions, and this process is shown to depend on YAP activity [19]. To study cell migration on heterogeneous matrices, gel systems with gradient stiffness have been used to show durotaxis

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for single [20] and collective cells [7], and define specific roles of myosin isoforms [21] in cell polarization during spontaneous migration across these substrates. However, it remains unknown whether the cells that are primed on a given ECM for a defined duration retain their mechanosensitive signatures even after moving to a new microenvironment. We refer to this persistent influence of cellular mechanosensitivity on cell migration as the 'mechanical memory of migratory cells'. In this study, we asked – do collectively migrating cells remember their past matrix stiffness as they move across mechanically dissimilar microenvironments?

To test this question, we developed a modular polyacrylamide (mPA) substrate comprising contiguous primary and secondary ECM regions of independently defined stiffness. We found that a monolayer of cells primed on a stiff ECM migrated faster and in a more coordinated manner after arriving on a soft secondary ECM, as compared to those cultured on a homogeneously soft ECM. Nuclear translocation of YAP persisted even after cells arrived onto a softer secondary ECM and shRNA-mediated depletion of YAP dramatically blunted this mechanical memory-dependent cell migration. Taken together, our results bring an additional dimension to the existing framework of mechanosensitive migration of epithelial cells in response to their current microenvironment. Mechanical memory in migratory cells may have a particular significance to cancer metastasis, where future invasion potential of escaped cancer cells may be predicted by exploiting their persistent dependency on the primary tumor microenvironment stiffness.

2. Materials and methods

2.1. Modular polyacrylamide (mPA) hydrogels

Contiguous polyacrylamide gels with distinct modules were fabricated through a step-by-step polymerization of PA solutions of defined compositions. Precursor solutions containing the acrylamide:bisacrylamide (A:B) percentages of 4:0.2% or 12:0.6% were mixed with 0.5% Ammonium Persulphate (APS) and 0.05% Tetramethylethylenediamine (TEMED) [22]. Red fluorescent carboxylate-modified beads of 200-nm diameter were added at 0.1% concentration to the stiff PA precursor solution to identify the interface between dissimilar ECM modules. Next, a volume of PA precursor solution sufficient to achieve a gel thickness of 100 μm was dispensed on the coverslip and covered with a glass slide of defined size to confine the spreading of the PA droplet in each module of the substrate. This step was repeated for all three modules (Fig. 1A) to fabricate the entire modular PA (mPA) hydrogel substrate. After polymerization, mPA gels were sterilized for 1 h under UV light. Subsequently, PA gels were treated with 0.5 mg/ml of sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino) hexanoate (Sulfo-SANPAH) (Thermo Fisher Scientific) prepared in 50 mM HEPES buffer (Santa Cruz Biotechnologies) and crosslinked to the mPA surface upon activation with 365 nm UV for 10 min. After extensive washing with 50 mM HEPES buffer, mPA gels incubated with 0.05 mg/mL of rat-tail collagen type I (Santa Cruz Biotechnologies) overnight at 4 °C.

2.2. Mechanical characterization of PA hydrogels

Atomic Force Microscopy (AFM) measurements of polyacrylamide gels were performed using an MFP-3D-BIO atomic force microscope (Asylum Research, Santa Barbara, CA). Olympus TR400PB AFM probes with an Au/Cr coated silicon nitride cantilever and pyramidal tip were used, with spring constants ranging from 20 to 30 pN/nm, as measured by thermal calibration. Force maps in square regions of 40 μm edge length, with 169 points per force map, were taken at equal spacing across the gels. Measurements were performed across at least 4 mm length on each side of the interface dividing the primary and secondary ECM regions, as shown in Fig. 1b. Elastic moduli were extracted from force curves using a modified Hertz model [23].

2.3. Cell culture and collective migration assay

Human mammary epithelial non-tumorigenic MCF10A cells were cultured in DMEM-F12 (GE Healthcare Life Sciences), supplemented with 5% horse serum (Invitrogen), 20 ng/mL epidermal growth factor (EGF, Miltenyi Biotec Inc), 0.5 mg/mL hydrocortisone (Sigma-Aldrich), 100 ng/mL cholera toxin (Sigma-Aldrich), 10 μg/mL insulin (Sigma-Aldrich), and 1% (v/v) penicillin-streptomycin (Sigma-Aldrich). Tumorigenic mammary epithelial MCF7 cells were grown in DMEM (Sigma-Aldrich) containing 10% fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin (Sigma-Aldrich), and 1% non-essential amino acids (0.1 mM). Human epidermoid carcinoma A431 cells were grown in DMEM (Sigma-Aldrich) containing 10% fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin (Sigma-Aldrich), 1% sodium pyruvate (Sigma-Aldrich), 1% sodium bicarbonate (Sigma-Aldrich), and 1% non-essential amino acids (0.1 mM). A PDMS stencil was designed and fabricated with a rectangular opening in the center, restricting the culture of

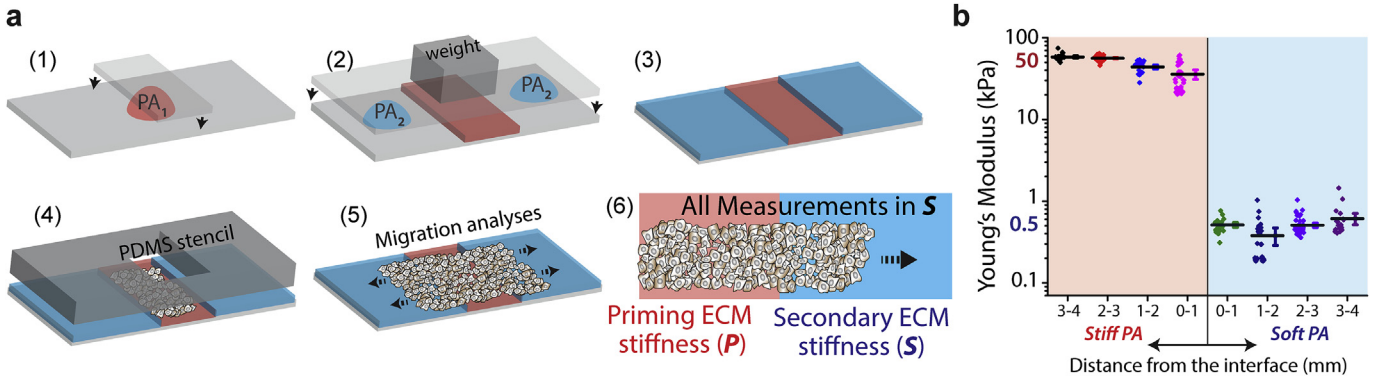

Fig. 1. Contiguous substrate with regions of dissimilar stiffness. (a) Schematic describing the fabrication steps of mPA substrates of heterogeneous stiffness through modular polymerization of PA solutions of distinct compositions, resulting in dissimilar ECM stiffness in adjoining primary and secondary regions. **(b)** Atomic Force Microscopy (AFM) measurements of Young's Modulus of PA gels plotted in logarithmic scale at different locations within a substrate with dissimilar primary and secondary ECM regions. Stiffness values are averaged over 1 mm length intervals and plotted along with scattered data points and error bars (SEM). N > 150. Data is included from at least 3 different PA gels, in which the left side was intended to be stiff (acrylamide/bisacrylamide = 12/0.6%) and right side as soft (acrylamide/bisacrylamide = 4/0.2%) matrix.