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A composite hydrogel platform for the dissection of tumor cell migration at tissue interfaces

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ABSTRACT

Glioblastoma multiforme (GBM), the most prevalent primary brain cancer, is characterized by diffuse infiltration of tumor cells into brain tissue, which severely complicates surgical resection and contributes to tumor recurrence. The most rapid mode of tissue infiltration occurs along blood vessels or white matter tracts, which represent topological interfaces thought to serve as "tracks" that speed cell migration. Despite this observation, the field lacks experimental paradigms that capture key features of these tissue interfaces and allow reductionist dissection of mechanisms of this interfacial motility. To address this need, we developed a culture system in which tumor cells are sandwiched between a fibronectin-coated ventral surface representing vascular basement membrane and a dorsal hyaluronic acid (HA) surface representing brain parenchyma. We find that inclusion of the dorsal HA surface induces formation of adhesive complexes and significantly slows cell migration relative to a free fibronectin-coated surface. This retardation is amplified by inclusion of integrin binding peptides in the dorsal layer and expression of CD44, suggesting that the dorsal surface slows migration through biochemically specific mechanisms rather than simple steric hindrance. Moreover, both the reduction in migration speed and assembly of dorsal adhesions depend on myosin activation and the stiffness of the ventral layer, implying that mechanochemical feedback directed by the ventral layer can influence adhesive signaling at the dorsal surface.

1. Introduction

Cell migration and the mechanisms that underlie specific migratory phenotypes are increasingly recognized to depend on extracellular context, especially the structure and mechanics of the extracellular matrix (ECM) [1–3]. On planar two-dimensional substrates, migration is typically described as being driven by a balance between actin polymerization at the cell front and actomyosin contraction at the cell rear that is transmitted to the ECM via adhesions [4]. In three-dimensional ECMs, migration can take various forms including mesenchymal migration (perhaps most analogous to classical two-dimensional migration) to amoeboid migration, which is less adhesion-dependent and leverages intracellular hydrostatic pressure generated by actomyosin contractility to extrude the cell body through matrix pores [5]. Importantly, the molecular mechanisms that control these migration modes are as diverse as the number of migratory phenotypes. In fact, many cells dynamically switch from one mode to another as they encounter and navigate different microenvironments, highlighting the importance of studying cell migration in culture systems that capture defining architectural features of tissue [6–8].

Cell migration is often guided by heterogeneous structures within the ECM; for example, a diverse variety of invasive solid tumors proceed along pre-existing anatomical structures [9–12]. Metastastatic tumor cells have been clinically observed to preferentially migrate in bone cavities or between adipocytes, suggesting that the topographies of these structures may facilitate tissue dissemination [10]. Migration in this context may be regarded as being "interfacial" in nature, in that cells translocate along a ventral two-dimensional surface while surrounded on their dorsolateral surface by an amorphous ECM of a different composition. Other examples of interfacial migration are tumor cells that migrate between bundles of myelinated axons and connective brain tissue [10,13].

A particularly important example of interfacial migration is the invasion of glioblastoma multiforme (GBM), the most common and deadly primary brain tumor. The extreme lethality of this malignancy is attributed in part to its diffuse and unrelenting infiltration of brain tissue, effectively precluding complete surgical resection

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[14]. GBM invasion patterns are unlike most other aggressive malignancies, in that GBM cells rarely intravasate and metastasize to distant tissues, instead remaining within the brain [14,15]. The pre-existing structures that guide GBM, collectively known as the secondary structures of Scherer, include the subpial space, white matter tracts, and vascular beds [16]. While these structures are widely acknowledged to facilitate invasive migration, relatively little is known about the biophysical and molecular mechanisms through which they do so. For example, cells migrating along vascular beds simultaneously experience strong integrin-based inputs via fibronectin and laminin in the vascular basement membrane [15] while also receiving adhesive inputs from hyaluronic acid (HA) in the brain parenchyma, which can be mediated by HA receptors such as CD44 and RHAMM [17,18]. There are also substantial biophysical asymmetries within this adhesive microenvironment, as vascular beds tend to be orders of magnitude stiffer than the surrounding parenchyma [19–21]. How these asymmetric signals are integrated to regulate migration in GBM remains unknown.

Despite the acknowledged importance of migration along asymmetric tissue interfaces in many tumors, comparatively little is known about the molecular mechanisms that underlie this process. The fact that migration mechanisms depend strongly on context has created an unmet need for experimental paradigms that recapitulate key aspects of these interfaces. To address this need, we developed a simple experimental system that features asymmetric ECM signals representative of the brain parenchyma–vascular interface, and used it to investigate molecular mechanisms of adhesion and motility.

2. Methods

2.1. HA-methacrylate synthesis

Methacrylated HA was synthesized as described previously [22]. Briefly, high molecular weight HA (66 kDa-90 kDa; Lifecore technologies) was dissolved at 1 wt% in deionized water, and then a six-fold molar excess of methacrylic anhydride (Sigma) was added dropwise to the solution on ice. The pH of the reaction was adjusted to a value greater than 8, where it was held for the duration of the experiment. The reaction was allowed to proceed overnight. HA-methacrylate was isolated by the addition of a five-fold volumetric excess of cold acetone to the reaction solution. This mixture was then centrifuged to recover the precipitate, which contained the HA-methacrylate. The precipitate was then dissolved in water, flash-frozen, and lyophilized.

2.2. Interfacial culture system formation

Initially, fibronectin-coated polyacrylamide surfaces were prepared as described previously [23]. After sterilization of the hydrogels, U373-MG, U87-MG, or U373-MG-U cells were plated on the gels and allowed to adhere overnight. 25 μl of an HA-methacrylate solution was then poured onto the cell and crosslinked in situ with the bifunctional dithiothreitol (DTT; Sigma) to form covalent cross-links among HA chains [24]. In HA-RGD formulations, cysteine-containing RGD peptide (Ac-GCGYGRDSPG-NH2; Anaspec) was first reacted with HA-methacrylate for 2 h, prior to gelation. All hydrogels consisted of 5 wt% HA-methacryate. The solution was immediately sandwiched with a glass coverslip and allowed to polymerize for 4 h at 37 °C, after which fresh medium was added.

2.3. Cell culture

U373-MG and U87-MG human glioblastoma cells were obtained from the University of California, Berkeley Tissue Culture facility and cultured as described previously [23] in DMEM (Invitrogen) supplemented with 10% Calf Serum Advantage (JR Scientific, Inc.), 1% penicillin-streptomycin, 1% MEM non-essential amino acids, and 1% sodium pyruvate (Invitrogen). Given the recent recognition that U373-MG likely share an origin with U251-MG cells [25], we also obtained early-passage U373-MG cells (Sigma), which we termed U373-MG-U, reflecting their derivation from the original University of Uppsala stocks [25].

2.4. Inhibition of cell contractility

Rho-associated kinase (ROCK) inhibitor Y-27632 (10 μM; Calbiochem), NMMII inhibitor blebbistatin (10 μM; Sigma), or myosin light chain kinase inhibitor (MLCK) ML-7 (1 μM; Calbiochem) was added to cells in interface culture after overnight incubation. Cells were incubated with the drug for at least 12 h prior to imaging.

2.5. Measurement of cell motility

Live-cell imaging was performed with a Nikon TE2000E2 microscope equipped with an incubator chamber for control of temperature, humidity, and carbon dioxide. After formation of the interfacial culture system as described above, phase-contrast images of cells were collected for at least 5 h with a 10 × objective. Nuclei were then tracked from one frame to another to yield instantaneous migration speeds, which were then averaged over the entire time course of the experiment to yield the migration speed of a cell.

2.6. Fluorescence microscopy

Mouse anti-vinculin primary antibody (Sigma) and AlexaFluor 546 goat anti-mouse secondary antibody (Molecular Probes) were used to visualize vinculin. Rat anti-CD44 primary antibody (Hermes-1, Pierce) and AlexaFluor 647 chicken anti-rat

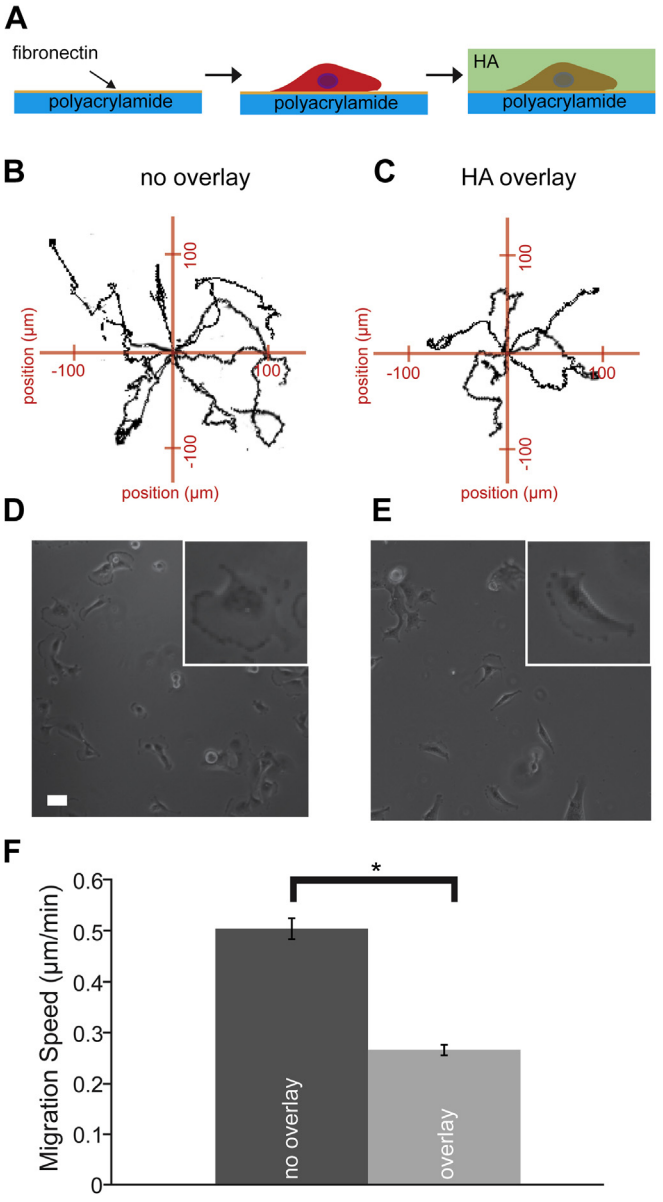

Fig. 1. Effects of HA overlay on cell migration. (A) Schematic of system. Initially, cells are seeded on defined-stiffness polyacrylamide hydrogels. After cell adhesion and spreading, soluble methacrylate-modified HA is cross-linked with DTT to form an insoluble network around the dorsolateral aspect of the cells. (B, C) Representative trajectories of single migrating cells over a 5 h period on a ventral fibronectin-coated polyacrylamide surface without (B) or with (C) a dorsal HA overlay present. (D, E) Phase contrast images of cells in these two configurations. (F) Quantification of migration speed under each condition. $N = 131, 125$ cells for overlay and no overlay, respectively. $^{*}P < 0.05$. Error bars are S.E.M. Scale bar is 50 μm.