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Phototriggered fibril-like environments arbitrate cell escapes and migration from endothelial monolayers

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ABSTRACT

Cell detachment and migration from the endothelium occurs during vasculogenesis and also in pathological states. Here, we use a novel approach to trigger single cell release from an endothelial monolayer by in-situ opening of adhesive, fibril-like environment using light-responsive ligands and scanning lasers. Cell escapes from the monolayer were observed on the fibril-like adhesive tracks with 3–15 μm width. The frequency of endothelial cell escapes increased monotonically with the fibril width and with the density of the light-activated adhesive ligand. Interestingly, treatment with VEGF induced cohesiveness within the cell layer, preventing cell leaks. When migrating through the tracks, cells presented body lateral reduction and nuclear deformation imposed by the line width and dependent on myosin contractility. Cell migration mode changed from mesenchymal to amoeboid-like when the adhesive tracks narrowed (≤5 μm). Moreover, cell nucleus was shrunk showing packed DNA on lines narrower than the nuclear dimensions in a mechanisms intimately associated with the stress fibers. This platform allows the detailed study of escapes and migratory transitions of cohesive cells, which are relevant processes in development and during diseases such as organ fibrosis and carcinomas.

1. Introduction

Endothelial cells are recognized as a source of cancer associated fibroblasts (CAF), accounting for the up to 40% of CAF found in tumors [55], in cardiac and pulmonary fibrosis [39] and tissue malformations [31]. Although this phenomenon is well documented, the critical factors that motivate and guide single cells to escape the endothelial monolayer, migrate into the stroma and transform into a different phenotype remain unclear.

Migration studies over the last decade have demonstrated that the molecular composition of the ECM and its architectural parameters like rigidity, density or topology of its constituting fibers are key variables of the migration environment [4,13]. Cells change their migration behavior to adapt to the different microenvironments using their contractile cytoskeleton in coordination with extracellular guidance cues. For simplicity, cell migration modes can be described as individual (i.e., amoeboid or mesenchymal) or collective (i.e., movement of coupled cells) migration [14]. In reality, cells adopt intermediate and interconvertible modes between states depending on the surrounding matrix.

Accruing evidence demonstrate that the topology of ECM fibers (diameter, aligned or random orientation) is a key parameter that forces cells to modify their migration properties [2–4,8,11,14,22,25,53,54]. Also, changes in ECM fibers occur under pathological conditions [12,14,29] and can trigger migratory transitions of embedded cells and modify invasive efficiency and metastasis occurrence [30]. Recent studies have shown that non-migrating cells undergoing a mesenchymal transition can

Abbreviations: CAF, cancer associated fibroblasts; ECM, extracellular matrix; FAs, focal adhesions; HUVEC, human umbilical vein endothelial cells; IS, instantaneous speed; nLA, nuclear long axes; nSA, nuclear short axes.

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experience a transition to amoeboid motility under confinement in a low adhesive environment [18,28]. Intermediate states between mesenchymal and amoeboid migration modes were observed upon changes in the concentration of adhesive ligands [28] or changes in the density, porosity and stiffness of the ECM during cancer progression [53].

Migration along aligned collagen fibers is crucial in tumor cell spreading [16,36], and aligned matrices were useful strategies for directing stem cell migration after transplantation into the rat auditory nerve and after spinal cord injuries [8,33,47]. Fiber-guided migration has been studied by seeding cells on planar substrates micropatterned with adhesive lines [8,9,32,38]. This simplified 1D microenvironment has been demonstrated to be an appropriate model to study cell migration along ECM fibers in 3D contexts, as it happens *in vivo* [36]. Escape and migration of cells out of a cohesive cell layer can also be studied in vitro by first spatially confining a group of cells and providing them free adhesive areas later. This is typically achieved using physical barriers, i.e. a PDMS block that confines cells in a reservoir and, when removed, allows cell expansion into new areas [10]. Physical barriers have also been combined with microcontact printed adhesive lines to expose cell in a monolayer to fiber-like adhesive tracks [50].

Whereas these methods have achieved remarkable results in explaining collective and single cell migration response in the new adhesive spaces, they have limited applicability to study the initial stages of the migration process, i.e. the cell–cell detachment event from the preformed layer and the cell changes when entering the confined 1D environment, and their dependence on geometrical and biochemical effectors. In this context, here we present a method that precisely regulates adhesive space in time and with defined geometries and ligand density, and offers constant monitoring of the cell escape process. It represents a new biomaterial platform that allows to phototrigger cell migration *in situ* [42]. It makes use of surface layers containing a photo-activatable adhesive peptide (cRGD) that binds integrin receptors at the cell membrane only after light exposure [52]. Localized exposure allows generation of cell-adhesive domains to which cells can attach and spread forming confined monolayers within a non-adhesive surrounding. In a second exposure step, adhesive microlines of desired geometry can be opened in-situ using a scanning laser. Here, we apply this methodology to study endothelial escapes and migration from a confined monolayer into fiber-like environments. We reveal the relevant role of the fiber diameter together with the adhesive ligand density in the activation of endothelial cell escapes as well as in their subsequent migration mode. Our studies provide insights on the role of ECM spatial organization in promoting cellular escapes and transitions modulated by the width of the fibrillary tracks, the density of the ligand and the presence of the external factors.

2. Results

2.1. Endothelial escapes can be photo-triggered in situ in the range of 3–15 μm linewidth

Patterned monolayers of endothelial cells were generated by culturing HUVECs on mask-irradiated substrates modified with photo-activatable RGD (Fig. 1a). The activated RGD pattern guided the formation of confined cell monolayers in the form of stripes. Cell monolayers remained confined to photo-activated patterns for more than 3 days under time lapse (movie 1), demonstrating that these substrates are suitable for long term microscopy studies. Migration lines were opened from the confined monolayers by scanning lines orthogonal to the pattern edge across the cell-free space using the 405 nm (5 mW) laser beam from the microscope (Fig. 1b). Cellular escapes from the monolayer and subsequent migration into adhesive lines of different widths was followed by time-lapse microscopy (Fig. 1c).

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Migration on lines wider than 15 μm mainly involved collective migration: the leader cell remained attached to the neighbors and protrusion of the monolayer was observed on the lines (Fig. 1c top panel), in agreement with previously reported data [42,50] and following a similar behavior recently described as epithelial bridges [49]. On lines narrower than 15 μm, cells spread through the entire width of the line, polarized, detached from the monolayer, and migrated as single cells (movie 2). No escape events were observed on lines narrower than 3 μm. These results demonstrate that the width of the adhesive path significantly influences endothelial escape, which occurs in our platform within the narrow range from 3 to 15 μm.

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2.2. Escaping cells form focal adhesion within the adhesive paths with fast front-rear aggregation

Single migrating cells strictly adapt their contour to the irradiated areas. Visualization of the focal adhesions (FAs) (cells transfected with GFP-vinculin plasmid) showed that mature FAs were only formed within the adhesive pathway (SI1e). Occasional spreading of the cell rim outside of the scanned areas was observed when transient blebs formed but disappeared soon afterwards because of lacking stable substrate interactions. In order to analyze the evolution of the adhesive contacts during cellular escapes, mature FAs were followed during single cell break into the lines by using a GFP-Vinculin construct. Fig. 1d and e shows confocal images of the FAs of a migrating cell from the endothelial monolayer to an open space or to a confined situation respectively. Distribution of the mature FAs was further quantified and analyzed by applying an aggregation index. Fig. 1f and g, show a heat map of the areas with highest vinculin density (red spots) corresponding to the location of the most mature FAs considered for analysis. A random distribution of FAs was found when cell migrated in an open space, with no particular FAs aggregation. In contrast, cells migrating in confined tracks showed aggregated FAs ($I \leq 0.05$), mostly accumulated at the front and at the rear.

2.3. Endothelial escape events are modulated by line width, ligand density and external factors

The number of escape events in which endothelial cells detached the confluent monolayer and entered to the migration lines was quantified (Fig. 2a and b). Endothelial escapes were scarce on 3 and 5 μm-width. In these cases, cells constantly attempted to enter the path, but most of them were unable to detach from the monolayer and initiate migration. The number of cell escapes into the adhesive lines increased monotonically with the line width (Fig. 2b), with significant difference between cells escaping on lines of 10 and 15 μm width and those on lines with 3 and 5 μm width. The density of adhesive ligand also affected the number of endothelial escapes (Fig. 2c). By increasing the exposure dose on the scanned spaces, a notable 2.5 fold increase in the number of escapes was observed was observed on lines of 10 μm. Cells were less sensitive to ligand density changes on lines of 5 μm-width, with a total increment of 0.9-fold within the tested density range. Notice that the line width became a more relevant factor to trigger cell escapes at increasing adhesive ligand density. The reduced cells sensitivity to ligand density on narrow lines is in agreement with