Probing Breast Cancer Cell Response to Heterogeneous Rigidity at the Nanoscale

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The ability to sense the rigidity of a cell's environment over a wide range of dimensions has recently been recognized as a important factor that influences cell function and behavior. Atypical response to rigidity is a a hallmark of transformed (cancerous) cells, a fact that is used on some diagnostic tests. Understanding the mechanisms that support cellular rigidity sensing can lead to new tissue engineering strategies and potential new therapies.

In this work, we adapt a technique have previously developed for the creation of biomimetic surfaces comprising regions of heterogeneous rigidity on the micro- and nanoscale. The surfaces are formed by exposing an elastomeric film of polydimethylsiloxane (PDMS) to a focused electron beam to form patterned regions of micro- and nanoscale spots. Finite element analysis of nanoindentation measurements performed on irradiated PDMS films show that in a thin layer near the film surface, where approximately 90% of the electron energy is absorbed, the Young's modulus of the elastomer undergoes an increase as a function of electron beam dose of up to two orders of magnitude (Fig. 1a). Surface chemical analysis indicates a depletion of oxygen in the exposed regions, rendering them more "glass-like," although there was little difference in surface energy (as measured by contact angle) between exposed and unexposed regions, and conditions were optimized to achieve uniform protein adhesion over the entire surface (Fig. 1b). Topographic measurements find shallow,gentle depressions (up to ~ 140 nm) with a large radius of curvature (~ 200 μm) in the exposed regions, resulting in a gently undulating surface (Fig. 1c).

Breast cancer cell lines MDA-MB231 and MDA-MB468, derived from human, triple negative and metastasized breast cancers were used. MB468 MTSs are surprisingly invasive in collagen gels despite having much lower integrin levels than the most aggressive MB231 cells (Fig. 2). Despite having low levels of beta1 integrin, MB468 cells invade 3D collagen matrices in the presence of strong force generation and ECM remodeling, which is unexpected for cells undergoing canonical amoeboid migration (Fig. 3). Both cell lines plated upon PDMS electron beam-exposed in a pattern of spots with diameters ranging from 2 μm to 0.25 μm displayed different co-localization of the intergrin α2β1 to the exposed features, depending on both rigidity and feature size. On spots with diameters of 2 μm, the integrin α2β1 appeared to accumulate on the features; For spots ~ 1μm and below this behavior was lost, and the cells appeared unable to identify the rigid regions (MB231, etc, Fig. 4a). MB468 cells have a stronger relative accumulation of integrins on stiff patches (in relation to the cell body in between the patches) than do MB231 cells (Fig. 4b), despite having fewer integrins. These results offer new clues into thr mechanisms of cancer cell invasiveness based on rigidity modulation.

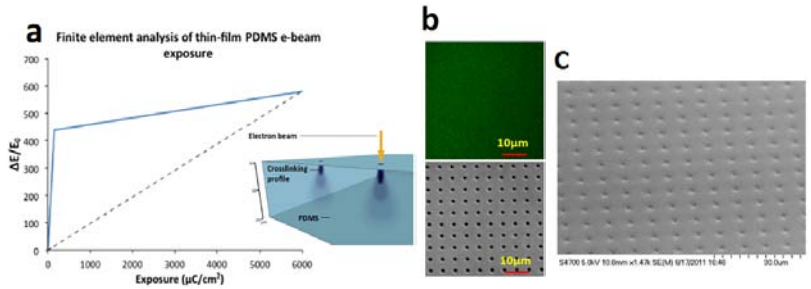

Fig .1 A composite with results on the characterization of the PDMS

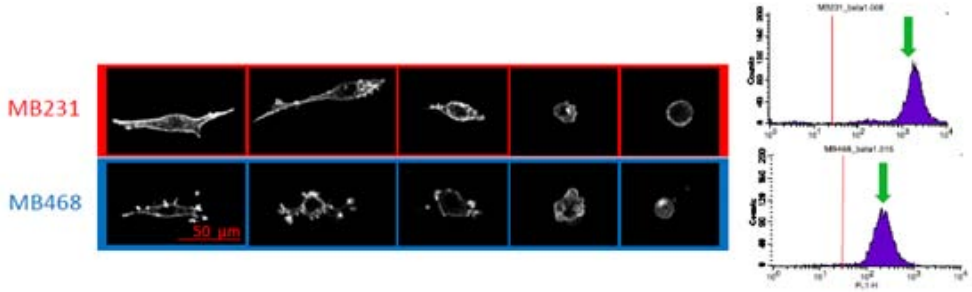

Fig. 2 MB468 have predominantly rounded morphology (80-95%) in all 3D matrices and a low expression level of beta1 integrin

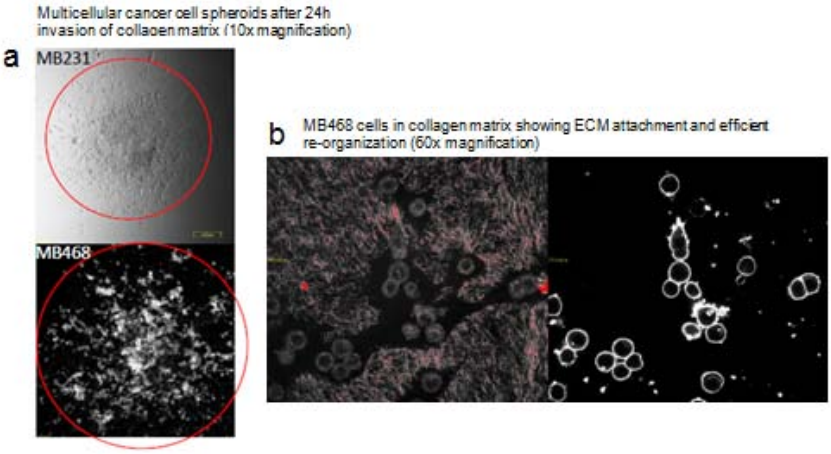

Fig. 3 Dissecting the mechanism of rounded cell migration

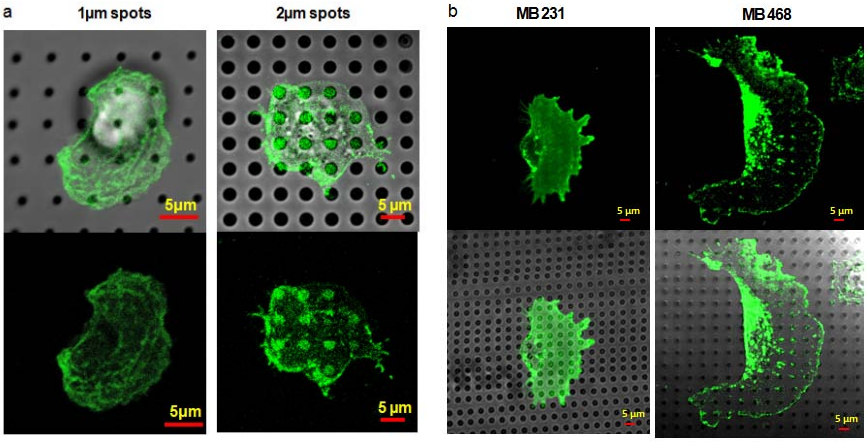

Fig. 4 MB231 and MB468 spreading on heterogeneous rigidity surfaces