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Research paper

Breast cancer cells mechanosensing in engineered matrices: Correlation with aggressive phenotype

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ABSTRACT

The pathogenesis of cancer is often driven by the modulation of the tumor microenvironment. Recent reports have highlighted that the progressive stiffening of tumor matrix is crucial for malignant transformation. Though extensive work has been done analyzing the mechanotransductive signals involved in tumor progression, it is still not clear whether the stiffness induced changes in cancer cell behavior is conserved across the invasive/aggressive phenotype of cells. Here, we used synthetic hydrogel based cell culture platform to correlate the aggressive potential of the breast cancer cells to the responses to matrix stiffness. The cellular functions such as proliferation, migration, and angiogenic capability were characterized. We report that the proliferation and motility of the highly aggressive cell line MDA-MB-231 increased with increase in matrix rigidity. We also demonstrated for the first time that the change in matrix stiffness stimulated the angiogenic activity of these cells as manifested from enhanced expression of vascular endothelial growth factor (VEGF). Inhibition of actomyosin contractility attenuated proliferation of MDA-MB-231 cells on stiff matrices while promoted the growth on soft gels. In addition, the release of VEGF was reduced upon inhibition of contractility. The less and non-aggressive breast cancer cells, SKBr3 and MCF-7 respectively displayed less dependency on matrix stiffness.

1. Introduction

It is well accepted in classical embryology that every cell type, from prokaryotes to multicellular organisms, can sense, assimilate, and integrate the changes in their microenvironment and subsequently alter their morphology, dynamic behavior, and eventually the cell fate (McBeath et al., 2004; Discher, 2010). Ample evidence implies that the alterations of the signaling pathways regulating cellular fate are not only dependent on the biochemical signals of the local microenvironments or niches but also on various physical cues including extracellular matrix (ECM) rigidity, porous architecture, and anisotropy that are generated and acted at the cell–ECM interface (Hoffman et al., 2011; Discher et al., 2005; Geiger

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et al., 2009; Huebsch et al., 2010). In the recent years, this concept of developmental biology has been increasingly accepted in cancer biology. The tumor microenvironment often differs from the normal tissue due to elevated production and deposition of collagen, altered organization or increased metalloproteinase (MMP) activity (Payne et al., 2007; Kessenbrock et al., 2010; Stronginin, 2006; Paszek and Weaver, 2004; Rozario and DeSimone, 2010). This perturbation in ECM dynamics has been implicated as a contributing factor in the development and progression of cancer (Levental et al., 2009; Paszek et al., 2005). In the context of breast cancer, it has been demonstrated that the compliance of the stroma within breast carcinomas is approximately 5–20 times more rigid than the normal breast tissue (Kass et al., 2007; Levental et al., 2009). Indeed, the initial screening of breast cancer lesions based on mammography and palpation identifies regions of stiff/dense tissue (Boyd et al., 1998). In a more recent study, increased matrix stiffness was observed in pre-malignant tissue and this increase was attributed to the malignant transformation in the breast (Levental et al., 2009).

Numerous studies have probed the connection between the altered ECM and malignant transformation of mammary epithelial cells (Bissell and Hines, 2011). Under basal conditions, non-malignant mammary epithelial cells form growth arrested acinar structures; however, alteration in matrix stiffness induces malignant phenotype including invasion of the basement membrane, uncontrolled proliferation, and loss of apicobasal polarity (Petersen et al., 1992; Debnath and Brugge, 2005; Weaver et al., 1997; Chaudhuri et al., 2014). The sensory machinery of cancer cells responds to the altered ECM dynamics by increasing actomyosin-mediated cytoskeletal organization to maintain the force equilibrium (Zhao et al., 2007). Rho GTPase has been identified as the key modulator of these processes with Rho activity being frequently elevated in tumor (Olson and Sahai, 2009). Biophysical studies have revealed that the activated Rho/Rho-associated kinase (ROCK) signaling pathway can influence tumor progression by regulating proliferation and invasion (Samuel et al., 2011). Despite the well-established association between tissue stiffness and tumor progression, it is still not clear whether the stiffness induced changes in cancer cell behavior is conserved across the invasive/aggressive phenotype of cells. Studies with large set of cancer cell lines demonstrated that majority of cancer cells display a preferential proliferation on stiff matrices while the remaining exhibited stiffness independent growth (Tilghman et al., 2012). However, the mechanisms underlying this mechanical preference are not known.

Tumor vascularization represents a hallmark of cancer; angiogenesis is a requisite not only for tumors to grow beyond 1–2 mm^3 and meet their insatiable metabolic demands (Folkman, 1974) but also provide a conduit for the cancerous cells to spread to distant organs (Fidler, 2003). Tumor vascularization is mediated by an overbalance of stimulatory angiogenic molecules including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), and angiopoientin (Ang1 and Ang2) released by the tumor cells. As a major component of local microenvironment, ECM and its fragments have emerged as an important contributor to the vascularization of tumors. For example, various ECM fragments including endostatin, tumstatin, canstatin, and hexastatin (derived from collagen) likely coordinate with other pro-angiogenic molecules including VEGF to initiate vascular branching (Weis and Cheresh, 2011). In addition, integrin clustering upregulates the expression of VEGF in cancer cells and subsequently enhances tumor vascularization via phosphorylation of p66 Shc (De et al., 2005). However, not much is known how alterations in ECM mechanics influence the expression of angiogenic factors by the cancer cells.

Most often hydrogels from natural ECM proteins such as collagen, fibrin, or matrigel have been utilized for studying cell–ECM interactions. However, while using these hydrogels the stiffness of the gels cannot be decoupled from other parameters such as ligand density, fiber thickness, and porous morphology (Discher et al., 2005; Trappmann and Chen, 2013). Therefore, parsing the contribution of matrix compliances on the structural and functional properties of cancer cells is difficult. On the other hand, the synthetic hydrogels such as polyacrylamide (PA) or polyethylene glycol (PEG) have inherent advantages such as well-defined mechanical properties and chemical inertness. These materials also provide a "blank" slate on which bioactive motifs can be easily incorporated (Lin and Anseth, 2011; Ceccarelli and Putnam, 2014). In this study, we manipulated the rigidity of PEG diacrylate (PEGDA) and gelatin methacrylate (GelMA) hybrid scaffolds by varying the crosslinking time. GelMA, obtained via conjugation of methacrylate groups to gelatin, provides the cell binding motifs e.g. RGD as well as matrix metalloproteinase (MMP) sensitive degradation groups (Kaemmerer et al., 2014). The stiffness of the matrices was varied from 16 kPa to 36 kPa to span the mechanical properties reported for cancerous breast tissue (Wellman et al., 1999). We evaluated the morphology/spread area, proliferation, migration, and angiogenic capability of the cancer cells seeded on substrates of different rigidities and investigated whether the breast cancer cells with different aggressive phenotype respond differently to changes in matrix mechanics. Our results indicate that the highly aggressive cell line, MDA-MB-231, displayed a preferential proliferation and migration on stiffer matrix. In addition, the increased stiffness stimulated angiogenic capability of MDA-MB-231 cells, as measured from elevated level of vascular endothelial growth factor (VEGF) released in conditioned media. On the other hand, even though proliferation of less invasive and non-invasive cell lines SKBr3 and MCF-7 respectively increased significantly when plated on the stiff matrices, matrix rigidity had no impact on their morphology, migration, and angiogenic potential. To assess whether alteration of cellular functions is mediated via Rho–ROCK signaling pathway, the cancer cells were challenged with pharmacological modulator of ROCK, Y-27632. Our data demonstrate that inhibition of actomyosin contractility stimulated the proliferation of cells on compliant matrices irrespective of aggressive phenotype. However, proliferation of MDA-MB-231 and SKBr3 was inhibited on stiffer matrices unlike the non-metastatic MCF-7 cells. Our study also revealed that modulation of contractile tension reduced the expression of angiogenic molecules by MDA-MB-231 cells.