Cell

Biomechanical Remodeling of the Microenvironment by Stromal Caveolin-1 Favors Tumor Invasion and Metastasis

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DOI 10.1016/j.cell.2011.05.040

SUMMARY

Mechanotransduction is a key determinant of tissue homeostasis and tumor progression. It is driven by intercellular adhesions, cell contractility, and forces generated within the microenvironment and is dependent on extracellular matrix composition, organization, and compliance. We show that caveolin-1 (Cav1) favors cell elongation in three-dimensional cultures and promotes Rho- and force-dependent contraction, matrix alignment, and microenvironment stiffening through regulation of p190RhoGAP. In turn, microenvironment remodeling by Cav1 fibroblasts forces cell elongation. Cav1-deficient mice have disorganized stromal tissue architecture. Stroma associated with human carcinomas and melanoma metastases is enriched in Cav1-expressing carcinoma-associated fibroblasts (CAFs). Cav1 expression in breast CAFs correlates with low survival, and Cav1 depletion in CAFs decreases CAF contractility. Consistently, fibroblast expression of Cav1, through p190RhoGAP regulation, favors directional migration and invasiveness of carcinoma cells in vitro. In vivo, stromal Cav1 remodels peri- and intratumoral microenvironments to facilitate tumor invasion, correlating with increased metastatic potency. Thus, Cav1 modulates tissue responses through force-dependent architectural regulation of the microenvironment.

INTRODUCTION

In vivo, cells interact with a three-dimensional (3D) microenvironment (Yamada and Cukierman, 2007). The mechanical force of these interactions, by altering tissue tension, acts as a molecular switch that determines cell fate (Engler et al., 2009). Tension generated in an extracellular microenvironment induces and cooperates with opposing forces applied by cells (mechanoreciprocity). In embryogenesis such tensile forces govern tissue organization (Krieg et al., 2008), and mammary acinar architecture relies on matrix compliance and cell tension (Ronnov-Jessen and Bissell, 2008).

Microenvironment-mediated tensile forces also contribute to disease. Matrix stiffness promotes breast cancer progression via mechanoreciprocal induction of Rho-dependent cell contractility (Levental et al., 2009). The microenvironment is also important for tumor invasion and metastasis: tumor cells (TCs) migrate along tracks made of extracellular matrix (ECM) collagen fibers (Friedl and Gilmour, 2009), and whereas reticular collagen surrounding mammary glands restrains invasion, Rho-mediated alignment of dense collagen fibers perpendicular to the tumor boundary promotes it (Provenzano et al., 2008). Activated fibroblasts facilitate tumor cell invasion through protease- and force-dependent generation of ECM tracks (Gaggioli et al., 2007). Carcinoma-associated fibroblasts (CAFs) and mesenchymal stem cells, via paracrine cytokine signaling, promote tumor growth, invasion, and metastasis (Karnoub et al., 2007; Orimo et al., 2005). Elucidation of tumor microenvironment remodeling mechanisms is thus an important area of research.

Caveolin-1 (Cav1), the major component of endocytic caveolae plasma membrane (PM) invaginations, has many functions outside caveolae (Parton and Simons, 2007). Cav1 activates

Rho by regulating its endogenous inhibitor p190RhoGAP (p190) and assists in focal adhesion (FA) stabilization required for directional cell migration (Goetz et al., 2008a; Grande-Garcia et al., 2007). The role of Cav1 in tumor progression remains unclear. In most primary tumors Cav1 levels decrease, allowing proliferation, anchorage independence, and angiogenesis, whereas metastasis correlates with Cav1 re-expression, promoting invasion, survival, and multidrug resistance (Goetz et al., 2008b). Most studies have focused on Cav1 expression in TCs, with little attention paid to a possible role in the tumor microenvironment. Because Cav1, via a mechanism involving Cav1 Tyr14 and p190, is essential for fibroblast function (Grande-Garcia et al., 2007), we investigated the role of fibroblast-Cav1 in stroma assembly.

We show that Cav1 regulates Rho GTPase activity by modulating membrane partitioning of p190 and thereby its phosphorylation. Fibroblast expression of Cav1 in vitro and in vivo favors an organized 3D stromal architecture that promotes spindle morphology, facilitates TC invasion, and increases p190-dependent metastatic potency. These findings correlate with increased numbers of Cav1-expressing CAFs in the stroma of human tumor samples. Cav1 silencing in human CAFs decreases their contractility, identifying a role for Cav1 in normal tissue homeostasis and pathological scenarios.

RESULTS

Cav1 Regulates Matrix-Induced Cell Morphology and Reciprocal Interaction with the 3D Microenvironment through Contraction

For a physiologically realistic culture substrate, we generated cell-free 3D matrices from confluent 8 day fibroblasts cultured in the presence of ascorbic acid; the resulting fibroblast-derived 3D matrices (FDMs) are rich in fibronectin (FN) and closely resemble in vivo mesenchymal matrices (Figure 1A, Figure S1A available online, and Movie S1). We seeded FDMs with Cav1 wild-type (Cav1WT) and Cav1 knockout (Cav1KO) mouse embryonic fibroblasts (MEFs) and analyzed cell morphology. Growth in FDMs doubled the cell length:breadth ratio (elliptical factor/EF) of Cav1WT MEFs (Figure 1B and Figure S1B) and almost halved their surface area compared with growth on 2D FN (Figure S1C). In contrast, FDM culture only mildly affected the morphology of Cav1KO MEFs, though it increased the number of cell protrusions (Figures 1B and 1C). Similar results were obtained when cells were grown in collagen-I (Col-I) gels (Figure 1D, Movie S2, and Movie S3).

Consistent with Cav1-dependent Rac regulation (Grande-Garcia et al., 2007), Cav1 deficiency increased PM targeting of Rac1 and its downstream effector phospho-S141-Pak1 (Figure 1E and Figure S1D). In contrast, phosphorylation of myosin light chain-2 (pMLC) was decreased (not shown), suggesting that Cav1 influences cell-induced matrix contractility. Consistently, Cav1WT MEFs contracted Col-I gels more effectively than Cav1KO MEFS at all cell concentrations tested (Figure 1F and Figure S1E). Re-expression of unmodified Cav1, but not its nonphosphorylatable mutant Cav1Y14F, rescued Rac1 localization, cell elongation, gel contraction, and pMLC levels in Cav1KO MEFs (Figure 1G and data not shown). Cav1 thus regulates features of fibroblasts that influence mechanical 3D microenvironment remodeling.

Cav1-Dependent Microenvironment Regulates Cell Shape, Protrusion Number, Rac1 Activity, and Morphology of Integrin-Dependent Adhesion Structures

To test the role of Cav1 in 3D microenvironment formation, we compared culture in FDMs produced from immortalized Cav1WT and Cav1KO MEFs with 2D culture. Three-dimensional growth raised smooth muscle actin (SMA) expression preferentially in WT MEFs close to levels in primary cultures (pMEFs) (Figure S2A). When these FDMs were reseeded with Cav1WT or Cav1KO MEFs, cells of both genotypes were more elongated when grown in FDMs generated by WT MEFs (Figure 2B and Figure S2B). These results suggest that lack of Cav1 alters FDM structure and composition; the most elongated cells were obtained when Cav1WT MEFs were plated in Cav1WT FDMs. Re-expression of Cav1 in Cav1KO MEFs rescued the ability to generate pro-elongation 3D matrices, whereas re-expression of Cav1Y14F had no effect (Figures 2C and 2D). Thus Cav1, through residue Tyr14, favors fibroblast elongation directly through endogenous expression and indirectly through cell-dependent 3D microenvironment remodeling, indicating that endogenous Cav1 and the Cav1-dependent microenvironment cooperate to enhance cell polarity. Cav1WT FDMs also reduced the number of cell protrusions (Figure 2E and Figure S2C) and Rac-GTPase activity (Figure 2F) independently of Cav1 expression by seeded cells, confirming the ability of Cav1-dependent ECM to favor in vivo-like spindle morphology.

We next assessed the impact of the Cav1-dependent microenvironment on the formation of 3D-matrix adhesions. Integrin-dependent 3D-matrix adhesions differ from regular FA in molecular composition (lower pY397FAK levels) and are longer (up to 19 μm) and thinner (Cukierman et al., 2001). Cav1WT and Cav1KO FDMs both decreased adhesion-targeted pY397FAK levels compared to a 2D FN substrate (Figure S2D). The longest 3D-matrix adhesions were obtained when Cav1WT MEFs were plated in Cav1WT FDMs (11.14 ± 0.48 μm) (Figure 2G and Figure S2E), indicating that both matrix and cells are important for determining adhesion length. The matrix adhesion marker vinculin localizes to 3D-matrix adhesions and its recruitment is force dependent (Cukierman et al., 2001), and the mobile vinculin fraction, determined by fluorescence recovery after photobleaching (FRAP), is an index of adhesion-dependent force increase (Bershadsky et al., 2003). Cav1KO FDMs increased the mobile vinculin fraction (Figures 2Ha–2Hc), indicating that these matrices slow the maturation of adhesion structures and the generation of adhesion-dependent forces. Endogenous Cav1 expression contributes little to the maturation of 3D-matrix adhesions (Figures 2Hb and 2Hc), contrasting the strong influence of the assorted FDMs.

Cav1 Promotes Patterning and Stiffness of 3D Matrices thus Regulating Normal Tissue Architecture

To assess the impact of Cav1 expression on microenvironment organization, compliance, and composition, we measured FN fiber orientation in FDMs. FN fibers in Cav1WT-derived FDMs were more parallel than in Cav1KO FDMs (Figures 3A and 3B).