

Mini Review

Regulation of the Microenvironment by Rho GTPase Signaling in the Epithelium: Implications for Breast Cancer Development and Progression

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Abstract

Deregulation of Rho GTPase expression and activity levels is found in a number of cancers, including breast cancers. Aberrant Rho GTPase signaling promotes tumorigenic behaviors in a cell-autonomous manner. The development of conditional knockout and overexpression mouse models of Rho GTPases and their regulators has allowed for investigation of the impact of aberrant Rho signaling in the context of the complex in vivo environment. These studies, including studies from our laboratory investigating the effects of Cdc42 and p190B RhoGAP overexpression in the developing mammary gland, indicate that altered Rho signaling in the epithelium impacts the microenvironment. We propose that hyperactivated Rho signaling in neoplastic cells may contribute to tumor formation by promoting the development of a pro-tumorigenic and pro-invasive microenvironment. The availability of conditional Rho GTPase mouse models will facilitate these studies in the future.

ABBREVIATIONS

Rho: Ras Homologous; GTPase: Guanosine Triphosphatase; RhoGAP: Rho GTPase activating protein; Cdc42: Cell Division Cycle 42; ECM: Extracellular Matrix; MMP: Matrix Metalloprotease; CAF: Carcinoma Associated Fibroblast; CXCL10: CXC Chemokine Ligand 10; IL-12: Interleukin-12; CSF-2: Colony Stimulating Factor-2; IFN: Interferon

INTRODUCTION

Breast cancer is the leading cancer diagnosis and the second leading cause of cancer related deaths among women in the United States [1]. The vast majority of breast cancer related deaths occur from distant metastases. While the 5-year survival rate of women diagnosed with localized breast cancer is 98.6%, this rate drops below 25% for women with distant metastases [1]. Therefore, inhibiting breast cancer development and progression to ultimately prevent metastasis offers the best hope of extending survival.

The development, progression, and metastasis of breast cancer require reciprocal tumor-stromal interactions. These

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interactions involve a complex interplay between tumor cells, fibroblasts, and immune cells. Secreted factors and mechanical signaling loops between these cells induce pro-tumorigenic and pro-invasive changes in the microenvironment characterized by increased Extracellular Matrix (ECM) deposition and remodeling as well as elevated expression of pro-tumorigenic growth factors and cytokines [2]. The effects of the microenvironment on breast tumor cell proliferation, invasion, metastasis, and chemoresistance have been intensely investigated [3]. Furthermore, increased infiltration and activation of fibroblasts and immune cells such as macrophages in human breast tumors correlate with poor prognosis [4,5]. However, the mechanisms by which neoplastic breast cells promote the development of the pro-tumorigenic and pro-invasive microenvironment are less well understood. Defining these mechanisms may provide new targets whose inhibition could serve to block development of the aberrant microenvironment or to restore the normal microenvironment, which naturally functions to restrain tumor formation and progression [6].

The Ras homologous (Rho) sub-family of Ras GTPases regulates a multitude of cellular processes that are important

for tumorigenesis and metastasis [7]. Elevated expression and activity levels of a number of Rho GTPases have been documented in many cancers, including invasive breast cancers [8-10]. While the cell autonomous effects of deregulated Rho signaling in cancer cells are fairly well understood [11], how aberrant Rho signaling impacts the microenvironment is now just beginning to be elucidated. This likely reflects the fact that for many years the effects of abnormal Rho signaling have been studied in isolated cells in culture. More recently, the development of conditional knockout and overexpression mouse models for several Rho family members and their regulators has allowed for investigation of the impact of altered Rho signaling in the context of the complex *in vivo* environment [12]. Furthermore, the increased use of three-dimensional culture models that mimic aspects of the connective tissue environment as well as heterotypic co-cultures have facilitated dissection of the cellular and molecular mechanisms governing epithelial-stromal cell cross-talk that disrupt epithelial architecture and proliferation control, and promote invasive behavior. Collectively, these models have provided tools to investigate the mechanisms by which altered Rho signaling in the epithelium may contribute to the development of the pro-tumorigenic and pro-invasive microenvironment.

Our laboratory has been interested in understanding how aberrant Rho signaling disrupts epithelial morphogenesis in the developing postnatal mammary gland as a first step towards understanding how it contributes to the development and progression of breast cancer. Most recently, we have focused on Cell Division Cycle 42 (Cdc42), one of the best-characterized members of the Rho GTPase family. Overexpression and hyperactivation of Cdc42 in human breast Invasive Ductal Carcinomas (IDC) has been demonstrated [9,10], and numerous *in vitro* studies and a few *in vivo* xenograft studies have demonstrated tumor cell autonomous functions for Cdc42 in breast tumor proliferation, migration, invasion, and metastasis [13-15]. Using a tetracycline (Tet)-regulatable Cdc42 mouse model that conditionally over expresses Cdc42 in the mammary epithelium, we have shown that Cdc42 overexpression during mammary gland development disrupts ductal morphogenesis leading to hyperbudded and trifurcated terminal end buds (TEBs, the structures that drive ductal elongation) and increased side branching [16]. Importantly, these ductal abnormalities were associated with stromal alterations reminiscent of an activated tumor microenvironment, including increased ECM deposition, elevated expression of matrix remodeling enzymes, and altered expression of pro-inflammatory cytokines (unpublished results).

A myriad of published studies investigating the effects of conditional knockout of Cdc42 have demonstrated cell type and tissue specific functions for Cdc42 in developing epithelia [17]. In addition to cell autonomous roles, loss of Cdc42 in some developing epithelia markedly impacts the adjacent microenvironment. For example, conditional knockout of Cdc42 in the developing epidermis results in epidermal hyperplasia in association with increased basement membrane protein deposition and altered epithelial-mesenchymal interactions [18]. Similarly, Cdc42 knockout in the developing lung impairs basement membrane deposition disrupting epithelial-stromal interactions [19]. Furthermore, in the developing pancreas

loss of Cdc42 in the developing epithelium disrupts cell fate specification in a non-cell autonomous manner by disrupting the microenvironment [20]. Together, these studies support the notion that Cdc42 signaling in the epithelium impacts the microenvironment in developing tissues.

Interestingly, there are several parallels between our studies investigating Cdc42 and our previous studies investigating the effects of overexpression of p190B Rho GTPase activating protein (RhoGAP), and inhibitor of Rho and Rac GTPases, in the developing mammary gland. P190B elicited a similar response when conditionally overexpressed in the developing mammary epithelium of a Tet-regulatable p190B mouse model. P190B overexpressing mammary glands exhibited a disorganized, hyperbranched ductal tree in conjunction with features of aberrant stromal activation, including increased ECM deposition and elevated expression of lysyl oxidase, a collagen cross-linking enzyme [21,22]. Collectively, these studies suggest that Rho GTPase signaling in the epithelium plays a substantive role in relaying signals to the adjacent microenvironment and that its misregulation can lead to stromal activation and phenotypes that are known to contribute to the disruption of normal tissue architecture and increase invasive behavior.

Studies have shown that the development of pro-tumorigenic and pro-invasive fibroblasts, carcinoma association fibroblasts (CAFs), occurs in a progressive manner where CAFs acquire increasingly pro-tumorigenic behaviors coincident with progression of mammary hyperplasia to adenoma to adenocarcinoma [23]. Interestingly, the pro-angiogenic and pro-invasive behaviors of CAFs can be blocked by Rho kinase and myosin inhibitors, indicating a key role for cytoskeletal tension in CAF behavior. Our published data indicate that epithelial overexpression of Cdc42 or p190B increases mammary epithelial cell contractility and activation of myosin, which correlated with increased production of ECM proteins and remodeling enzymes in the developing mammary gland [16,22]. These studies suggest that deregulated Rho GTPase activity in epithelia may trigger activation of stromal fibroblasts in part by increasing tissue tension.

In addition to CAFs, the importance of macrophages during breast cancer development, invasion, and metastasis is well known [24]. Furthermore, macrophage infiltration and activation correlates with poor prognosis in breast cancer patients [5,25]. Data indicate that macrophages express pro-inflammatory cytokines during early stages of mammary tumorigenesis and switch to an anti-inflammatory phenotype during later stages of mammary tumorigenesis [26,27]. While a number of studies have provided insight into the mechanisms by which anti-inflammatory macrophages promote later stages of tumor progression, less is known about the contribution of pro-inflammatory macrophages to the development and progression of early stage lesions. Our unpublished results indicate that stromal cells isolated from mice overexpressing Cdc42 in the developing mammary epithelium rapidly upregulate several markers of pro-inflammatory macrophages (e.g. CXCL10 and IL-12) as well as cytokines that activate pro-inflammatory phenotypes in macrophages (e.g. CSF-2 and INF- γ) following just

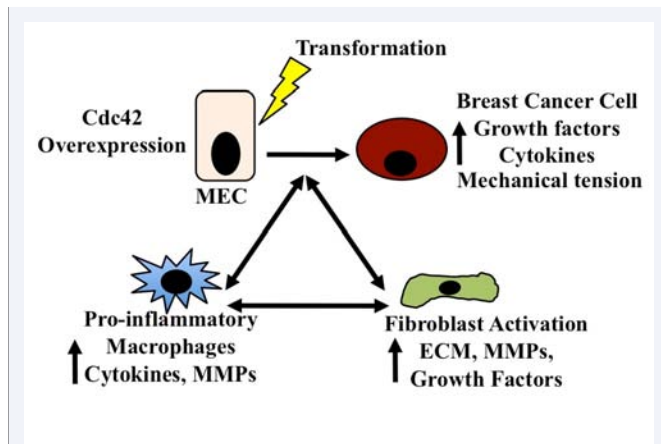


Figure 1 Epithelial Cdc42 overexpression induces a pro-tumorigenic microenvironment that may contribute to the development and progression of breast cancer.

Studies using conditional Cdc42 overexpressing mice have revealed that Cdc42 overexpression in the developing mammary gland induces aberrant epithelial morphogenesis and hyperbranching in association with increased ECM deposition and expression of collagen, MMPs, and pro-inflammatory cytokines [16]. Secreted and mechanical signals between the neoplastic epithelium and stromal cells likely contribute to these pro-tumorigenic and pro-invasive changes in the microenvironment.

one week of Cdc42 overexpression in the mammary epithelium. In addition, Matrix Metalloprotease (MMP) gene expression, in particular MMP-3, is rapidly upregulated in stromal cells in response to Cdc42 overexpression in the epithelium [16]. Stromal cells, including fibroblasts and macrophages, produce MMP-3, and it is a well-known inducer of secondary and tertiary branching in the developing postnatal mammary gland [28]. Thus, upregulation of stromally derived MMP-3 may be one mechanism underlying the disruption of epithelial architecture that occurs in response to Cdc42 overexpression.

In conclusion, our data and studies from other laboratories indicate that the Rho signaling network within epithelia regulates signaling loops that affect fibroblast and macrophage behavior in the adjacent microenvironment. It is attractive to speculate that Cdc42 overexpression and hyper activation in pre-neoplastic and transformed cells may drive tumor development and progression in part by inducing pro-tumorigenic and pro-invasive changes in the microenvironment (Figure 1). While the tumor cell autonomous actions of several Rho GTPases, including Cdc42, are well known [11,13], the contribution of Rho GTPase overexpression and hyper activation in tumor cells to regulation of macrophage and fibroblast activities within the tumor microenvironment remains to be determined. Recently, there have been significant advances towards developing specific inhibitors that target Cdc42 [29,30]. Thus, targeting Cdc42 or its downstream effectors may provide a unique opportunity to simultaneously inhibit both the tumor cells and the tumor microenvironment. A deeper understanding of the contribution of epithelial Cdc42 overexpression to the development of the tumor microenvironment will facilitate the development of effective breast cancer therapeutics that target these crucial

tumor-stromal interactions.

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