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Collagen density regulates xenobiotic and hypoxic response of mammary epithelial cells

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ABSTRACT

Breast density, where collagen I is the dominant component, is a significant breast cancer risk factor. Cell surface integrins interact with collagen, activate focal adhesion kinase (FAK), and downstream cell signals associated with xenobiotics (AhR, ARNT) and hypoxia (HIF-1α, ARNT). We examined if mammary cells cultured in high density (HD) or low density (LD) collagen gels affected xenobiotic or hypoxic responses. ARNT production was significantly reduced by HD culture and in response to a FAK inhibitor. Consistent with a decrease in ARNT, AhR and HIF-1α reporter activation and VEGF production was lower in HD compared to LD. However, P450 production was enhanced in HD and induced by AhR and HIF-1α agonists, possibly in response to increased NF-κB activaton. Thus, collagen density differentially regulates downstream cell signals of AhR and HIF-1α by modulating the activity of FAK, the release of NF-κB transcriptional factors, and the levels of ARNT.

1. Introduction

Breast cancer is a worldwide clinical problem amounting to approximately 1.38 million diagnoses and 450,000 deaths each year (Ferlay et al., 2010). Various risk factors have been identified in the development of breast cancer including increasing age, high breast density, nulliparity, obesity, hormone replacement therapy, alcohol consumption, early age of menarche, late age of menopause, and radiation exposure (Dumitrescu and Cotarla, 2005). Of these factors, high breast density has been indicated to be one of the greatest independent risk factors across various breast cancer subtypes (McCormack and dos Santos Silva, 2006; Phipps et al., 2012). Histological examination of dense and normal breast tissue has revealed that collagen is a primary component of dense breast tissue (Guo et al., 2001). The increased presence of type I collagen has also been clinically linked to metastatic tumors via genetic based analyses of tumor biopsies (Ramaswamy et al., 2003), suggesting that cellular responses to collagen may be linked to tumorigenesis.

Collagen is an extracellular matrix (ECM) protein known to interact with cell surface integrins in mammary gland development and tumor formation (Keely, 2011). The protein is

Abbreviations: AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; DFOM, deferoxamine mesylate salt; ECM, extracellular matrix; FAK, focal adhesion kinase; HD, high density; HIF, hypoxia inducible transcription factor; HRE, hypoxia response element; LD, low density; VEGF, vascular endothelial growth factor; XRE, xenobiotic response element.

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an established component of normal breast architecture and the dominant component of dense breast tissue, a significant breast cancer risk factor (Guo et al., 2001; McCormack and dos Santos Silva, 2006; Phipps et al., 2012). We have previously shown that increased stromal collagen in mouse mammary tissue significantly increases tumor formation and metastases (Provenzano et al., 2008b). Moreover, mammary cells cultured in stiff collagen matrices exhibit mechanosignaling events that regulate gene expression and subsequent cellular differentiation and proliferation (Schedin and Keely, 2011).

Signaling through focal adhesion kinase (FAK) is a significant signaling pathway by which cells respond to dense collagen matrices (Provenzano et al., 2009). This tyrosine kinase localizes at contact points where cell surface integrins interact with components of the ECM, and plays a critical role in the downstream processes of cell spreading, adhesion, motility, survival and cell cycle progression (Golubovskaya and Cance, 2010). FAK is also implicated in breast tumorigenesis, particularly in mouse models where tissue-specific knock-out of FAK in the mammary gland significantly diminishes tumor formation and the development of cancerous hyperplasias (Lahlou et al., 2007; Provenzano et al., 2008a; Pylayeva et al., 2009). Microarray analyses of the benign tumors arising in FAK knock-out mammary glands identified several genes that had previously been associated with a metastasis signature (Wang et al., 2002; Provenzano et al., 2008a). Among mRNAs decreased in tumors lacking FAK, we identified AhR, HIF-1α and ARNT for further investigation as possible transcriptional regulators of breast cancer progression.

Hypoxia inducible transcription factors (HIF-1α, HIF-1β) dimerize and activate downstream genes in promoting aerobic glycolysis and tumorigenesis (Curran and Keely, 2013; Morandi and Chiarugi, 2014). Overexpression of HIF-1α has been identified in primary breast cancers and murine models where increased production of vascular endothelial growth factor (VEGF) is also identified (Kimbro and Simons, 2006; Stein et al., 2009; Curran and Keely, 2013). HIF-1β, which is a dimer partner to HIF-1α, is also known as ARNT (aryl hydrocarbon receptor nuclear translocator) and a dimer partner to the aryl hydrocarbon receptor (AhR) in xenobiotic metabolism. Xenobiotic ligands in the cytoplasm bind AhR which induces the release of AhR from a multiprotein complex and allows the receptor to translocate to the nucleus, dimerize with ARNT and activate phase enzymes involved in the efflux of the chemical/ligand (Chen et al., 2012b). In breast cancer, dysregulation of AhR and particular phase I enzymes have been associated with increased tumorigenesis (Dialyna et al., 2001; Goode et al., 2013).

AhR is mostly commonly known for ligand induced activation in response to polycyclic aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Additionally, AhR is known to bind to the endogenous ligand, kynurenine, which is up-regulated in breast cancer (Mezrich et al., 2010; Lyon et al., 2011; Tang et al., 2014). Certain drugs used in the therapeutic treatment of breast cancer in human studies or murine models include microtubule-interfering agents, tamoxifen, doxorubicin, VEGF antagonist SU5416, and tranilast, which are all also known AhR ligands (Overmoyer et al., 2007; Vrzal et al., 2008; DuSell et al., 2010; Prud'homme et al., 2010; Volkova et al., 2011; Mezrich et al., 2012). Both AhR and HIF-1α regulate genes involved in the assembly and maintenance of the ECM (Kung et al., 2009; Gilkes et al., 2014). These transcription factors are also known to exhibit possible crosstalk via the shared dimer component ARNT (Chan et al., 1999). In the current study, we investigated whether changes in the collagen matrix alter the responses of normal mammary gland cells to the ARNT-coupled pathways involving AhR and HIF-1α.

2. Materials and methods

2.1. Cell lines and cell culture

The MCF-10A cell line was used as representative models of normal breast epithelia (Debnath et al., 2003). The MCF-10A cell line was obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in stiff (3 mg/ml) or compliant (1 mg/ml) type I collagen gels as previously described. In brief, rat tail collagen (Corning, 354249, Bedford, MA) was mixed thoroughly with a neutralization solution (1:2) containing 100 mM Hepes and 2× PBS (pH 7.4) and placed on ice for no more than 10 min. High density (3 mg/ml) or low density (1 mg/ml) collagen in neutralized solutions were added to cells suspended in complete media. Complete media contained 50% DMEM with high glucose (Gibco Life Technologies, Grand Island, NY), 50% F-12 Nutrient mix (Gibco), 5% horse serum (Gibco), 10 μg/ml bovine insulin (Cell Applications, Inc., San Diego, CA), 0.5 mg/ml hydrocortisone (Sigma-Aldrich, St. Louis, MO), and 20 ng/ml epidermal growth factor (EMD Millipore/Calbiochem, Darmstadt, Germany). Cells and media were thoroughly mixed with neutralized collagen. A total volume of 1.5 ml cells/media/collagen was aliquotted to one well of a 6-well sterile non-tissue culture treated plate (BD Falcon™, Franklin Lakes, NJ) and incubated at room temperature for 10 min. The plate was transferred to a 5% CO_2, 37 °C incubator for 2 h. The gels were then released from the plate by gently sliding a 200 μl pipette tip around the outer edge of the gel and 2 ml of complete media was added to the gel prior to returning the plate to a 5% CO_2, 37 °C incubator. In some experiments, cells were treated with 100 μM deferoxamine mesylate salt (DFOM, Sigma), 100 μM tranilast, 10 μM FAK ATP inhibitor PF-562271 (Selleckchem, Houston, TX) or 10 μM Src kinase inhibitor PP2 (Calbiochem/Millipore, Billerica, MA).

2.2. Reporter assay

The luciferase plasmids containing the hypoxia response element (HRE) or xenobiotic response element (XRE) were kind gifts from Dr. Christopher A. Bradfield, University of Wisconsin-Madison and the Renilla plasmid was a kind gift from Dr. Michele A. Wozniak, University of Pennsylvania. Cells (5×10^5/5 ml) were plated in a 60 mm tissue culture dish (BD Falcon™, Franklin Lakes, NJ) for 24 h and transfected with 2 μg of luciferase plasmid, 1 μg of Renilla plasmid and 12 μl of Lipofectamine® 2000 Transfection Reagent (Invitrogen Life Technologies, Carlsbad, CA). After transfecting 24 h, cells were lifted and cultured in collagen gels ±100 μM deferoxamine mesylate salt (Sigma) or 200 μM tranilast (Sigma) for an additional 24 h. Gels were rinsed with