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Presentation

Pressure stimulates breast cancer cell adhesion independently of cell cycle and apoptosis regulatory protein (CARP)-1 regulation of focal adhesion kinase

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Abstract

Background: Pressure stimulates colon cancer adhesion via focal adhesion kinase (FAK). Extracellular pressures reaching 29 mm Hg have been reported in rapidly growing breast cancers, and tumors experience pressure during surgical manipulation. We hypothesized that pressure stimulates breast cancer adhesion and that CARP-1, which influences cancer biology, inhibits FAK, and modulates pressure effects.
Methods: We compared MDA-MB-468 breast cancer cells under ambient or 15-mm Hg increased pressure. We studied FAK-397 autophosphorylation, which parallels activation, after CARP-1 overexpression, and investigated whether CARP-1 stable overexpression or reduction alters pressure-stimulated adhesion.
Results: Pressure increased MDA-MB-468 adhesion 25% ($n = 30$, $P < .05$). CARP-1 overexpression inhibited FAK-397 phosphorylation. However, pressure stimulated adhesion equivalently in CARP-1–overexpressing and CARP-1–reduced lines ($n = 6$, $P < .05$).
Conclusions: Pressure within proliferative tumors or during manipulation may activate breast cancer cells. Thus, inhibiting pressure signaling in rapidly growing breast tumors may be beneficial. CARP-1 does regulate FAK, but CARP-1 modulation does not alter pressure-stimulated adhesion. Targeting CARP-1 is unlikely to manipulate this pathway.

Keywords: Adhesion; Cancer; FAK; Pressure; Signal transduction

Malignant tumor cells in cancer patients undergoing surgical resection may be susceptible to extracellular forces such as increased pressure and shear stress during tumor manipulation or during passage through the venous and lymphatic system during dissemination. Tumor cells shed into a body cavity may also be exposed to pressure, shear, and turbulence during laparoscopic insufflation or irrigation, whereas intraabdominal pressure increases substantially for 2 to 3 days after surgery because of bowel edema and third spacing [1,2]. Previous studies suggest that increases in extracellular pressure, similar in magnitude to those observed in vivo in some surgical patients, stimulate the adhesion of colon cancer cells by a focal adhesion kinase (FAK)-dependent mechanism, but the other mediators of this pathway are poorly understood [3].

Interstitial fluid pressure in patients with invasive ductal carcinoma is increased compared with normal breast parenchyma and other malignant and nonmalignant conditions [4,5] by as much as 29 mm Hg. We hypothesized that the adhesion of breast cancer cells might therefore be stimulated by extracellular pressure in a manner similar to that observed in colon cancer cells.

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CARP-1/CCAR1 is a novel protein that has been implicated in cell-cycle and apoptosis regulation [6,7]. CARP-1 is a perinuclear/cytoplasmic transducer of apoptosis signaling that binds with stratifin/14-3-3 sigma. Depletion of CARP-1 inhibits apoptosis caused by agents such as adriamycin. Conversely, overexpression of CARP-1 increases apoptosis and reduces expression of various cell-cycle regulators including c-myc and cyclin B. CARP-1 is expressed in a variety of cell types including human breast and colon cancer cells. Because CARP-1 regulates diverse intracellular signaling pathways, we hypothesized that CARP-1 levels influence pressure-stimulated cancer cell adhesion by modulating mediators of the pathways of cell adhesion and motility such as FAK [6].

We tested these hypotheses by studying MDA-MB-468 breast cancer cells in which CARP-1 expression was downregulated or enhanced by stable expression of antisense or full-length complementary DNA (cDNA), respectively. We performed adhesion assays under conditions of ambient or 15 mm Hg increased extracellular pressure and assessed CARP-1 levels, CARP-1-dependent attenuation of FAK activation, and CARP-1 regulation of FAK by Western blotting.

Materials and Methods

Cells

We used estrogen receptor–negative, p53-negative MDA-MB-468 HBC cells for this study because they were used in the initial characterization of CARP-1 and were subsequently used in all of our previous studies involving CARP-1. These cells were cultured and maintained by using Dulbecco's modified Eagle medium, Ham's F-12 medium, and fetal bovine serum as previously described [8,9].

Transfections

The MDA-MB-468 HBC cells were transfected with vector plasmids pcDNA3, pcDNA3/Hygro, pcDNA3/Hygro CARP-1 antisense clone 1.6, or pcDNA3/CARP-1-myc-His clone 6.16. For stable transfections, this was followed by selection by using 800 μg/mL of neomycin or 400 μg/mL of hygromycin. From this, multiple resistant stable sublines were generated and propagated. Generation and propagation of retroviruses encoding wild-type myc-His-tagged CARP-1 and transduction of HBC cells has been described previously [6,7].

Pressure regulation

Pressure was applied in an airtight box with inlet and outlet valves and a pressure gauge within a warming incubator as previously described [3,10]. The box was prewarmed to 37°C in an effort to prevent internal fluctuations. The temperature was maintained to within $\pm$2°C and the pressure to within $\pm$1.5 mm Hg. Control cells were maintained at ambient pressure within the same incubator.

Adhesion assays

Cells were plated at 100,000 per well in 6 well plates for both the cells exposed to increased pressure and the control cells. All plates were precoated with collagen I (Sigma, St Louis, MO) in an enzyme-linked immunosorbent assay–based coating buffer as previously described [11]. One plate was placed in 15 mm Hg or 30 mm Hg at 37°C for 30 minutes, whereas the other was simply placed in 37°C under ambient pressure conditions for 30 minutes. At the end of 30 minutes, nonadherent cells were gently washed away with phosphate-buffered saline, and adherent cells were fixed with formalin, stained with hematoxylin, and counted in 20 individual, random, high-power fields per well by using an Olympus microscope (Center Valley, PA).

Western blotting

For studies of signal transduction, cells were lysed in modified Laemmli buffer including protease inhibitors (50 mmol/L Tris, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L ethylene glycol tetraacetic acid, 1% Triton X100, 1% deoxycholic acid, 10% glycerol, 10 mmol/L NaPyrPO4, 50 mmol/L NaF, 2 mg/mL aprotinin, 2 mg/mL leupeptin, 1 mmol/L phenylmethanesulfonyl fluoride, 1 mmol/L Na_3VO_4, 0.1% SDS) at 4°C and assayed for protein by the bicinchoninic acid assay (Pierce, Rockford, IL). Proteins were then resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis; transferred to nitrocellulose membranes; and subjected to Western blotting for CARP-1, FAK-397, FAK, and α-tubulin as a protein-loading control. Antibody to CARP-1 was generated (Sigma Genosys, The Woodlands, TX) by immunizing rabbits as previously described [6,9]. Antibodies to FAK-397 and total FAK protein were obtained from Biosource International (Camarillo, CA). After washing and exposure to appropriate secondary antibodies, proteins were visualized by ECL Plus technique (Amersham, Arlington Heights, IL) and imaged and quantitated by using a Kodak Phosphorimager (Perkin Elmer, Boston, MA) for visualization and denisitometric analysis software. All blots studied were within the linear range of exposure.

Statistical analysis

Statistical analysis was done by using SigmaStat (SPSS, Inc, Chicago, IL). Paired and unpaired *t* tests were used for this purpose as appropriate, seeking 95% confidence.

Results

We previously performed various experiments involving different pressures and different time points and found that a 30-minute exposure to increased pressure was sufficient to both activate intracellular signals and enhance adhesion in colonic adenocarcinoma and other cancer cells [3]. We chose to replicate these conditions for the present study both for consistency with our previous signal transduction work and because breast cancers are likely to experience a range of increased pressures up to 30 mm Hg; 15-mm Hg pressure increases therefore seem relevant to breast cancer pathophysiology.

Breast cancer cells display increased adhesion in response to increased extracellular pressure

We first sought to determine whether adhesion of MDA-MB-468 breast cancer cells increases in response to pressure because pressure effects on breast cancer cells had not previously been shown. Single-cell suspensions of several subclones of MDA-MB-468 breast cancer cells were allowed to adhere to type I collagen for 30 minutes under