Down-regulation of BRCA2 Expression by Collagen Type I Promotes Prostate Cancer Cell Proliferation

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BRCA2 is a tumor suppressor gene that when mutated confers an increased susceptibility to developing breast and prostate carcinoma. Besides its role in mediating DNA repair, new evidence suggests that BRCA2 may also play a role in suppressing cancer cell growth. Because altered interactions between neoplastic cells and the surrounding extracellular matrix (ECM) play a pivotal role in unchecked cancer cell proliferation and metastatic progression, we hypothesized that the ECM may have an effect in BRCA2 expression. By using normal and prostate carcinoma cell lines, we demonstrated that although normal cells transiently increase BRCA2 protein levels when adhering to the ECM protein collagen type I (COL1), carcinoma cells exhibit a significant reduction in BRCA2 protein. This aberrant effect is independent from de novo protein synthesis and requires dynamic collagen COL1-β1 integrin signaling through phosphatidylinositol 3-kinase leading to BRCA2 ubiquitination and degradation in the proteasome. Blocking or inhibiting collagen COL1-mediated DNA synthesis and proliferation after cancer cell adhesion to ECM. Interference assays triggered new DNA synthesis, a trophic effect that is abrogated by recombinant BRCA2 protein synthesis and results from unliganded β1 integrin signaling through phosphatidylinositol 3-kinase and has no effect in BRCA2 expression. By using normal and prostate carcinoma cell lines, we demonstrated that although normal cells transiently increase BRCA2 protein levels when adhering to the ECM protein collagen type I (COL1), carcinoma cells exhibit a significant reduction in BRCA2 protein. This aberrant effect is independent from de novo protein synthesis and requires dynamic collagen COL1-β1 integrin signaling through phosphatidylinositol 3-kinase leading to BRCA2 ubiquitination and degradation in the proteasome. Blocking or inhibiting collagen COL1-mediated DNA synthesis and proliferation after cancer cell adhesion to ECM. Interference assays triggered new DNA synthesis, a trophic effect that is abrogated by recombinant BRCA2 protein synthesis and results from unliganded β1 integrin signaling through phosphatidylinositol 3-kinase and has no effect in BRCA2 expression. 

Inactivation of the murine homologue Brca2 leads to embryonic lethality (8–11), and Brca2-null murine embryonic fibroblasts exhibit spontaneous accumulation of chromosomal abnormalities (12, 13) and defective cytokinesis (14). In addition to its role in mediating DNA repair and genome stability, new evidence suggests that BRCA2 could suppress tumor development by inhibiting cancer cell growth (15).

The majority of breast, ovarian, and prostate cancer cases is sporadic (16, 17). Despite the contribution of mutational inactivation of BRCA2 is rare, as it would require both gene copies to be affected. In contrast, BRCA2-containing familial breast and sporadic breast and ovarian cancers represent a major fraction of cases during cancer development and cell cycle regulation of BRCA2-null cells, as in other eukaryotic cells (18).

Several important questions still need to be answered, including the role of BRCA2 and inactivation and invasion of cancer cells. Adhesion through modulation of ECM expression may be involved in the preferential metastasis of prostate cancer to the bone has been hypothesized to involve collagen type I (COL1) (20), the major structural component of the bone (22). Collagens and other ECM proteins interact with cells through integrins (23), a family of heterodimeric transmembrane receptors composed of an α and a β subunit (24). These interactions activate cytoplasmic kinases, such as members of the mitogen-activated protein kinase/extracellular signal-regulated kinase-1 and -2 (25, 26) and the phosphatidylinositol (PI) 3-kinase/AKT (27, 28), which modulate the expression of genes exerting stringent control on cell proliferation (23).

In this study, we have elucidated a novel mechanism whereby adhesion of prostate carcinoma cells to the ECM protein COL1 results in BRCA2 protein degradation, which stimulates cancer cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Culture—PNT1A cells (a human prostate normal cell line established by immortalization of normal adult prostate epithelial cells),

*This work was supported by the MURST-PPRST Cluster 03 Grant and the MIUR-Contributi Straordinari di Ricerca/Aree Obiettivo 1 Grant (to E. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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PC-3 cells (a human prostate carcinoma cell line derived from a bone metastasis), and MCF-7 (a human breast adenocarcinoma cell line) were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK). PNT1A and PC-3 cells were kept in culture as described previously (29). MCF-7 cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% inactivated fetal bovine serum (BioSpa, Milan, Italy), 2 mM glutamine (Invitrogen), 100 units/ml penicillin (Invitrogen), and 100 mg/ml streptomycin (Invitrogen), at 37 °C in the presence of 5% CO2.

RNA Extraction and RT-PCR—Cells were grown to 60–70% confluence and washed twice with cold phosphate-buffered saline (PBS), and total cellular RNA was isolated using the TRIZol reagent (Invitrogen) following the manufacturer’s instructions. The amount of RNA in the present was measured by semiquantitative reverse transcription. Total RNA was reverse-transcribed, and cDNA was amplified by PCR using the Titan One Tube RT-PCR system (Roche Applied Science). The bands were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide.

Immunoblotting Analysis and Immunoprecipitation—Following harvest, cells were processed for immunoblotting. Membranes were probed with antibodies against the following proteins: p110 (H-239; Santa Cruz Biotechnology), GAPDH (H-136; Santa Cruz Biotechnology), BRCA2 (H-300; Santa Cruz Biotechnology), tubulin (H-9262; Chemicon), and p16 (H-9252; Santa Cruz Biotechnology). Immunoreactive bands were quantified using the Bio-Rad Dc protein assay reagent (Bio-Rad) according to the manufacturer’s protocol.
for 16 h at 4 °C in Tris-buffered saline, pH 7.5, 150 mM NaCl, 0.1% Tween 20). The membranes were then washed three times in TBS-T at room temperature. After three washes in TBS-T, proteins were visualized using the enhanced chemiluminescence (ECL) kit (Amersham Biosciences) according to the manufacturer's instructions. Densitometric values for immunoreactive bands were quantified using a GS-700 Imaging densitometer (Bio-Rad).

To perform a coupled immunoprecipitation-immunoblotting assay, the whole cell extract (0.25–0.5 mg of protein) was first precleared and then incubated overnight at 4 °C with 1 μg of polyclonal antibody to BRCA2 (H-300). Immunocomplexes were recovered by binding to protein A-Sepharose (Sigma) and washed five times with PBS containing 1% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 5 mM sodium pyrophosphate. Immunocomplexes were analyzed by 6% SDS-PAGE under reducing conditions followed by transfer to polyvinylidene difluoride membrane. Filters were immunoblotted using a 1:500 dilution of monoclonal antibody to ubiquitin (Sigma) or 2 μg/ml monoclonal antibody to BRCA2 (clone 5.23; Chemicon) following the manufacturer's instructions.

Cell Adhesion—Cell adhesion assays were carried out using 96-well tissue culture plates. Plates were pre-coated with different concentrations of COL1 (0.1–10 μg/ml) or P4G11 (0.5–1 μg/ml) for 16 h at 4 °C. Coating with 10 μg/ml bovine serum albumin (BSA; Sigma) was performed as a negative control. Coating with 10 μg/ml PLL was performed as a positive control. Cell adhesion was quantified by measuring the absorbance at 630 nm in a microtiter plate immunosorbent assay reader. Inhibition assays were performed by incubating cells for 1 h on ice in the presence of either human monoclonal antibody to human β1 integrin (ascites 1:200), or PLL (ascites 1:200) as a negative control. Triplicate observations were performed in each experiment.

Plasmid Expression Vectors—Expression vectors for wild-type PI 3-kinase p85 regulatory subunit (wt-p85), Δp85, a dominant negative form of PI 3-kinase, and p110*, a constitutively active form of PI 3-kinase, were generously provided by Dr. Robert Freeman (University of Rochester, Rochester, NY). The expression vector containing the wild-type BRCA2 cDNA was a kind gift of Dr. M. C. Hung (University of Texas M. D. Anderson Cancer Center, Houston). pCMV-β-gal vector expressing β-galactosidase was from Stratagene.

Transient Transfection—PNT1A and PC-3 cells were seeded into 6-well dishes at 4–5 × 10⁵ cells/well. After 24 h, transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The DNA sequence against which small interfering RNA (siRNA) for BRCA2 was created is AACACAGAUTAC-GAAGCAACAG (32). Duplexed RNA oligomer for BRCA2 was synthesized by Qiagen (Milan, Italy). Double-stranded RNA was transfected into the cells as 0.3 μg of siRNA/well. pCMV-β-gal (0.5 μg/well) was transfected along with 4.5 μg/well of either one of the PI 3-kinase variant cDNAs constructs or the empty vector (pcDNA3). Cells were harvested 36–96 h after transfection and used in immunoblotting, immunoprecipitation, and [3H]thymidine incorporation assays. Transfected cells were also plated in 48-well plates and stained for β-galactosidase expression to determine transfection efficiency. The β-galactosidase staining was performed as described by Manes et al. (33).

[3H]Thymidine Incorporation—Thymidine incorporation assay was used to measure the cell proliferative index. Cells were starved for 24 h, detached, plated in a 96-well plate (10,000 cells/well), and grown for 1C10 (ascites 1:200), or COL1 at 37 °C for the indicated times, washed, lysed, and processed for immunoblotting. Membranes were probed with anti-BRCA2 antibody. The blots are representative of three independent experiments.
COL1 Suppresses BRCA2 Expression in Cancer Cells

15 h either on PLL, PLL (10 μg/ml), COL1 (10 μg/ml), or P4G11 (1 μg/ml)-coated wells. Where indicated, cells were preincubated for 1 h on ice with P4C10 (ascites 1:200) or 1C10 (ascites 1:200) as a negative control, or added with MG132 (10 μM), or preincubated for 1 h at 37 °C with wortmannin (0.1 μM) or the solvent alone (MeSO) before plating. During the last 3 h of the 15-h culture, cells were pulsed with 1 μCi/ml methyl-3H]thymidine (Amersham Biosciences), washed three times with PBS at 0 °C, and dissolved with 10% SDS. Radioactivity was measured by using a scintillation counter (Beckman Instruments).

Statistical Analysis—Data are reported as the mean ± S.E. Statistical analysis was performed by the Student’s t test. All experiments were repeated at least twice.

RESULTS

In this study a normal human cell line (PNT1A), established by immortalization of normal adult prostate epithelial cells, non-tumorigenic in mice (34), and the prostate cancer epithelial cell line PC-3, derived from a bone metastasis (35, 36), were used as the experimental model. Both PNT1A and PC-3 cells expressed comparable levels of BRCA2 mRNA and protein as measured by RT-PCR (Fig. 1A) and Western blot assays (Fig. 1B), respectively. MCF-7, a breast cancer cell line that expresses wild-type BRCA2 (37), was included as a positive control (Fig. 1, A and B).

Cancer Cells Adhere More Efficiently to COL1 than Normal Prostate Cells—We proceeded to investigate the adhesive properties of PNT1A and PC-3 cells to COL1. Adhesion to BSA and PLL, an integrin-independent adhesive substrate, served as negative and positive controls, respectively. Serial dilutions were performed with the coating substrates, and the concentration that supported maximal adhesion, reported previously, was used for all subsequent experiments. Both cell lines displayed comparable adhesion to PLL (Fig. 2A) and COL1 (Fig. 2B), with no significant differences observed when interacting with PLL or COL1 at 4°C or 37°C. In general, COL1 adhesion was 2- to 3-fold higher than PLL adhesion. Because cell adhesion to COL1 is mainly mediated through integrin receptors, we next investigated whether the cell lines expressed similar levels of COL1-binding integrins. Adhesion with P4C10, a blocking antibody to β1 integrin, was completely depleted them after 4 h. BRCA2 protein levels did not change by >2-fold with PLL (Fig. 3A). However, adhesion to COL1 transiently abated BRCA2 mRNA to 35% and 12% of basal levels after 2.5 and 6 h, respectively, and remained elevated at 6 h. In PC-3 cells, although adhesion to PLL did not affect BRCA2 mRNA levels, adhesion to COL1 transiently abated BRCA2 mRNA to ~10% after 2.5 h and then to later increase to ~65% of basal levels after 6 h. However, the increase in mRNA 6 h after adhesion (Fig. 4A) did not result in an increase in BRCA2 protein (Fig. 3A). These results suggested that post-transcriptional mechanisms might be mainly responsible for BRCA2 protein depletion by COL1 in PC-3 cells.

We next proceeded to investigate whether de novo protein synthesis and/or protein degradation contributed to the reduction in BRCA2 protein levels in PC-3 cells after adhesion to COL1. Cycloheximide treatment of PC-3 cells did not prevent BRCA2 loss within 12 h of adhesion to COL1, indicating that de novo protein synthesis was not necessary for BRCA2 depletion (Fig. 4B). The involvement of lysosomal-, calpain-, and ubiquitin-proteasome-mediated proteolysis was investigated by measuring BRCA2 protein levels after allowing PC-3 cells to adhere to COL1 in the presence of the lysosomal inhibitor leupeptin, the calpain inhibitor ALLN, or the proteasome inhibitor MG132 (Fig. 4B). MG132 prevented BRCA2 depletion almost completely, with levels that were 89 ± 7% after 12 h of adhesion. Treatment of PC-3 cells with leupeptin failed to...
prevent BRCA2 depletion. Likewise, pretreatment with the lysosomal inhibitor pepstatin or the lysosomotropic agent NH$_4$Cl yielded similar results (data not shown). ALLN had a minor effect in preventing BRCA2 depletion, with levels of 18% after 12 h. Cell viability, as judged by trypan blue exclusion, was >98% in all the experiments, and cell incubation with the solvent Me$_2$SO did not affect BRCA2 protein levels.

To investigate whether BRCA2 is targeted for proteasome degradation through ubiquitination, we allowed PC-3 cells to adhere to COL1 in the presence of MG132 or solvent alone, and cell extracts were immunoprecipitated with an antibody to BRCA2, followed by Western blotting with an antibody against ubiquitin (Fig. 4C). Before adhesion (0 h), PC-3 cells showed minimal BRCA2 ubiquitination. After 3 h of adhesion to COL1, BRCA2 ubiquitination increased steadily at a pace that mirrored the reduction in BRCA2 protein levels seen in Fig. 3A. Treatment with MG132 further increased the levels of ubiquitinated BRCA2.

**PI 3-Kinase Activation after PC-3 Cell Adhesion to COL1 Promotes BRCA2 Ubiquitination**—Integrin engagement has been shown to trigger the activation of the PI 3-kinase signaling pathway (27, 28), which can modulate gene expression (23). Thus, we asked whether PI 3-kinase/AKT mediated the down-regulation of BRCA2 after PC-3 cell adhesion to COL1. The PI 3-kinase activity was measured by Western blotting with anti-phospho-Ser-473 AKT antibody following adhesion of PNT1A and PC-3 cells to COL1 or PLL (Fig. 5A). Both PNT1A and PC-3 cells showed basal AKT phosphorylation. Whereas PNT1A cells exhibited a 3.8-fold increase in AKT phosphorylation at 2 h of adhesion to COL1. These high levels remained unchanged throughout the 6 h of cell adhesion (Fig. 5A) and after 12 h of adhesion (data not shown). On the contrary, there was no change in AKT phosphorylation after PC-3 cell adhesion to PLL. The increase in AKT phosphorylation upon PC-3 cell adhesion to COL1 was dependent on $\beta_1$ integrin, as P4C10 pretreatment resulted in $\sim$80% inhibition as compared with 1C10-treated cells (Fig. 5B). To investigate whether activation of the PI 3-kinase/AKT was involved in the down-regulation of BRCA2 expression, we determined the effect of PI 3-kinase inhibitors on BRCA2 protein levels. In PC-3 cells, inhibition of the PI 3-kinase activity through wortmannin (Fig. 6A) or LY294002 (data not shown) caused a 3.8-fold increase of BRCA2 expression after 2.5 h of adhesion to COL1 but had no significant effect in PNT1A cells. After 6 h, BRCA2 levels were slightly higher (~23%) than those observed before cell attachment (0 h). Treatment with wortmannin did not affect BRCA2 protein levels in PC-3 cells adherent to PLL. As expected, wortmannin or LY294002 significantly inhibited AKT phosphorylation both in PNT1A and PC-3 cells (data not shown). These results were further validated by transiently transfecting PC-3 cells with either a dominant negative (Δp85) or a constitutively active (p110*) form of the PI 3-kinase before adhesion to COL1. Transfection with Δp85 completely prevented BRCA2 protein degradation, with 97 ± 5% of starting levels at 12 h (Fig. 6B).
transfections with p110* (Fig. 6B) or a wild-type form of the p85α subunit of the PI 3-kinase (data not shown) did not affect BRCA2 depletion by COL1. Overall, these results provided compelling evidence that β1 integrin signaling via the proteosome inhibitor MG132 (10 μM) or the p85α subunit of PI 3-kinase p85 (Δp85), or constitutively active PI 3-kinase p110 subunit (p110*). Thirty-six hours after transfection, the cells were allowed to adhere to COL1 at 37 °C for the indicated times in the presence or absence of the proteosome inhibitor MG132 (10 μM), or the solvent alone (MeSO). We first addressed the possibility that β1 integrin signaling via the proteosome inhibitor MG132 (10 μM) or the solvent alone (MeSO) and then lysed. Cell lysates were processed for immunoblotting with an antibody to BRCA2 and then immunoblotted with a monoclonal antibody to ubiquitin or to BRCA2 (C). The blots are representative of two independent experiments.

To investigate whether PI 3-kinase/AKT activation, and the increase in DNA synthesis after PC-3 cell adhesion to COL1, we measured [3H]thymidine incorporation following 12 h of cell adhesion to PL, PLL, or COL1. As shown in Fig. 7C, DNA synthesis increased by 54% upon 12 h of cell adhesion to COL1 compared with PLL or PL, but decreased by 39% after adhesion to COL1 (p < 0.001) (Fig. 7C, top panel). Treatment of BRCA2 overexpressing PC-3 cells with MG132 did not affect BRCA2 protein levels upon adhesion to PLL (data not shown) but resulted in a 210% increase upon adhesion to COL1 (p < 0.001) (Fig. 7C, top panel). The expression of recombinant BRCA2 protein did not affect [3H]thymidine incorporation in PC-3 cells adherent to PL or PLL but completely abrogated the increase in DNA synthesis in response to cell adhesion to COL1 (Fig. 7C, bottom panel). Consistently, treatment with MG132 did not affect DNA synthesis in BRCA2-transfected cells adherent to PLL but decreased [3H]thymidine incorporation in control and BRCA2 transfectants adherent to COL1 by 46 and 57%, respectively (p < 0.003).

Inhibition of PI 3-Kinase/AKT Abrogates COL1-β1 Integrin Stimulatory Effect on DNA Synthesis in PC-3 Cells—In order to establish whether a link exists between β1 integrin, PI 3-kinase/AKT activation, and the increase in DNA synthesis after PC-3 cell adhesion to COL1, we measured [3H]thymidine incorporation in PNT1A and PC-3 cells either preincubated with P4C10 before adhesion to COL1 or allowed to adhere to P4G11 (Fig. 8A) and in cells allowed to adhere to COL1 for 12 h in the presence or absence of wortmannin (Fig. 8B). Blocking of β1 integrin by P4C10 decreased by 27% the incorporation of [3H]thymidine in PC-3 cells (p < 0.01). Conversely, PC-3 cell
adhesion to a β1 integrin antagonist reduced DNA synthesis by 36% of [3H]thymidine incorporation in PC-3 cells adherent to COL1 by 46% ([H]thymidine as described in A). The effect was expressed as mean ± S.E. of triplicate wells. A representative experiment of three is shown.

DISCUSSION

We have uncovered evidence suggesting that ECM proteins enriched at metastatic bone sites have a stimulatory effect on prostate cancer cell proliferation. This proliferative effect is linked to BRCA2 protein depletion via β1 integrin-dependent activation of the PI 3-kinase pathway, which promotes BRCA2 ubiquitination and degradation in the proteasome.

Prostate carcinoma has an exquisite tendency to metastasize to the bone with as many as 90% of patients with advanced disease suffering from osseous metastasis (38). The selective nature of this process is suggestive of a unique microenvironment that provides prostate cancer cells with the factors necessary to sustain metastatic capabilities. The non-mineral osseous ECM consists mostly of COL1, a protein that serves as a substrate through interaction with αβ1, αβ1, and β1 integrins. Prior work has suggested that PC-3 cells, which are derived from a human bone metastasis (35), adhere to the collagenous component of the osseous matrix to a great extent through the αβ1 integrin (20). We observed that PC-3 cells exhibited an increased adhesion to COL1 compared with the normal prostate cell line PNT1A. This property hinged on the presence of β1 integrin as it was entirely prevented by a β1 integrin blocking antibody. It has been postulated that elevated cell adhesion to COL1 may partly explain the osteotropism displayed by metastatic prostate tumor cells (20, 39). Interestingly, both normal and cancer cells displayed comparable amounts of β1 integrin protein. Thus, it is conceivable that the different adhesive properties of PNT1A and PC-3 cells to COL1 could be because of the differential expression of specific α integrin subunits between the two cell types (40).

In addition to providing osteotropic properties to prostate cancer cells, we show for the first time that β1 integrin-mediated adhesion to an ECM protein has a negative effect on BRCA2 protein levels. Although in normal cells the adhesion to PLL and COL1 elicits a transient increase in BRCA2 protein, PC-3 cells exhibit a complete loss of BRCA2 after adhesion to COL1 but not to PLL. A few hours after adhesion, there is a sizable decrease in BRCA2 mRNA levels, which should contribute at least initially to lowering BRCA2 protein levels. How-
Among the latter, calpain- and ubiquitin-mediated proteolysis may have an analogous effect. A similar finding has been reported for BRCA1, a tumor suppressor gene functionally related to BRCA2 (42), after adhesion of breast carcinoma cells to laminin-1 and collagen type IV (43). Thus, it is conceivable that BRCA1 and BRCA2 proteins are at the cross-roads of abnormal cancer cell response to signaling from the extracellular environment.

We proceeded to dissect the biological mechanisms involved in BRCA2 disappearance in response to PC-3 cell adhesion to COL1. It had been reported previously that the BRCA2 protein depletion that occurs after ultraviolet irradiation requires new protein synthesis (44). However, adhesion experiments in the presence of the translational inhibitor cycloheximide failed to prevent BRCA2 degradation, implicating a more conventional proteolytic process. Two major proteolytic systems exist in mammalian cells, the lysosomal and the non-lysosomal systems (45); our experiments excluded any involvement of the former in the COL1-mediated depletion of BRCA2 protein. Among the latter, calpain- and ubiquitin-mediated proteolysis are likely to occur through a p53-independent mechanism. Furthermore, the increase in BRCA2 levels after cancer cell adhesion to COL1 is not exclusively by a COL1-β1 integrin mechanism. The PI 3-kinase/AKT signaling pathway (50, 51) has been shown to play a role in down-regulation of BRCA2 expression in response to mitomycin C-induced DNA damage (49) but not in ultraviolet irradiation-mediated BRCA2 depletion (44), suggesting that the mechanisms of regulation of BRCA2 expression are complex and may vary in different physiopathological conditions. Calpain-mediated proteolysis of BRCA2 protein was observed in our conditions but to a much lesser extent than proteolysis mediated by the ubiquitin-proteasome system.

Ligation of β1 integrins can activate both the mitogen-activated protein kinase/extracellular signal-regulated kinase and the PI 3-kinase/AKT signaling pathway (50, 51). In normal cells, the transient increase in BRCA2 expression after attachment to an adhesive substrate is accompanied by a transient increase in extracellular signal-regulated kinase phosphorylation. In contrast, PC-3 cell adhesion to COL1 triggers a conspicuous increase in AKT phosphorylation along with the decrease in BRCA2 levels. These two events were proven to be linked to each other in experiments whereby chemical inhibition of AKT phosphorylation with wortmannin or LY294002 or transfection of a dominant negative form of PI 3-kinase had the opposite effect and promoted BRCA2 ubiquitination and degradation upon PC-3 cell adhesion to COL1. These experiments provide compelling evidence that the PI 3-kinase

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2 L. Moro, A. A. Arbini, E. Marra, and M. Greco, manuscript in preparation.

3 L. Moro, A. A. Arbini, E. Marra, and M. Greco, unpublished data.
pathway promotes ubiquitination of BRCA2 protein in cancer cells, thus targeting it to the proteasome for proteolysis. Recent studies have shown that the PI 3-kinase/AKT pathway can control the expression of proteins such as tuberin, FOXO, p53, androgen receptor, and insulin receptor substrate-1 through ubiquitination (52–55). AKT has been shown to activate the ubiquitin ligase activity of murine double minute-2 that has been implicated in AKT-stimulated increase in p53 and androgen receptor degradation (53, 54, 56). However, the mechanism whereby PI 3-kinase/AKT targets substrates such as BRCA2 for ubiquitination remains to be investigated.

In normal prostate cells we did not find any consistent correlation between cell proliferation and the transient increase in BRCA2 expression resulting from attachment to an adhesive substrate. Adhesion to PLL diminishes the proliferative index, which could be rather attributed to the absence of specific mitogenic signals derived from the ECM (57). On the contrary, in cancer cells we demonstrate that BRCA2 protein degradation after adhesion to the ECM results in a conspicuous increase in DNA synthesis, an index of increased cell proliferation. This was corroborated by demonstrating that BRCA2 protein depletion after transfection with small interfering RNA did enhance DNA synthesis on COL1, and this effect was reversed by the expression of recombinant wild-type BRCA2 or by incubating the cells with a proteasome inhibitor. An increase in cell proliferation after PC-3 cell adhesion to COL1 had been noted in a previous report that showed an increase in the number of PC-3 cells after attachment to COL1 (39). Moreover, our findings are in line with previous reports showing down-regulation of BRCA2 mRNA expression during the proliferative expansion of a nodal marginal zone B-cell lymphoma (58), and inhibition of the neoplastic proliferation of prostate cancer cell lines expressing BRCA2 (59).

A number of studies have implicated COL1 receptors, in having a regulatory role (50, 59, 60). Others have shown that PI 3-kinase signaling is required for COL1 (61). Finally, it has been demonstrated that invasiveness is associated with the expression of COL1 (62–64). The results presented here play a central role in broadening the understanding that COL1 not only serves as a major adhesive substrate for prostate cancer cells but also provides a permissive substrate for enhanced cancer cell proliferation at metastatic osseous sites, a paramount event in the natural history of prostate carcinoma. Further elucidation of the regulatory mechanisms involved in BRCA2 expression by environmental signals will provide a better understanding of the molecular pathways that contribute to prostate cancer progression and metastasis.

Acknowledgments—We are grateful to Dr. M. C. Hung for the BRCA2 cDNA and Dr. R. Freeman for the PI 3-kinase constructs.

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J. Biol. Chem. 2005, 280:22482-22491.
doi: 10.1074/jbc.M414091200 originally published online April 1, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M414091200

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