Published in final edited form as:

Mol Carcinog. 2012 June ; 51(6): 500–507. doi:10.1002/mc.20813.

Transcriptional Down-Regulation of Brca1 and E-cadherin by CtBP1 in Breast Cancer

Yu Deng^{1,#}, Hui Deng^{1,2,#}, Jing Liu¹, Gangwen Han², Stephen Malkoski², Bolin Liu², Rui Zhao³, Xiao-Jing Wang^{2,*}, and Qinghong Zhang^{1,2,3,*}

¹ Department of Dermatology, University of Colorado, Denver, Aurora, CO 80045

² Department of Pathology, University of Colorado, Denver, Aurora, CO 80045

³ Department of Biochemistry and Molecular Genetics, University of Colorado, Denver, Aurora, CO 80045

Abstract

Carboxyl-terminal binding protein 1 (CtBP1) is a transcriptional co-repressor with oncogenic potential. Immunohistochemistry (IHC) staining using human breast cancer tissue arrays revealed that 92% of invasive ductal breast cancer cases have CtBP1-positive staining compared to 4% CtBP1-positive in normal breast tissue. To explore the functional impact of CtBP1 in breast cancer, we examined CtBP1's transcriptional regulation of known tumor suppressors, breast cancer susceptibility gene 1 (*Brca1*), and E-cadherin. We found CtBP1 was recruited to the promoter regions of *Brca1* and *E-cadherin* genes in breast cancer cells. Concomitantly, *Brca1* loss was detected in 57% and E-cadherin loss was detected in 76% of human invasive ductal breast cancers, and correlated with CtBP1 nuclear staining in these lesions. Importantly, siRNA knock down of CtBP1 restored *Brca1* and *E-cadherin* expression in breast cancer cell lines, implying CtBP1 down-regulates *Brca1* and *E-cadherin* genes in human breast cancer. This study provides evidence that although genetic loss of *Brca1* and E-cadherin are infrequent in breast cancer, they are downregulated at the transcriptional level by CtBP1 expression. Thus, CtBP1 activation could be a potential biomarker for breast cancer development.

Keywords

Brca1; CtBP1; E-cadherin; transcription; breast cancer

Introduction

CtBP1 was initially recognized as an adenoviral E1A-binding protein and was later identified as a transcriptional co-repressor of multiple tumor suppressors [1]. CtBP1 has been shown to suppress transcription of several tumor suppressors such as E-cadherin [2] [3,4] and p16INK4a [5]. Conversely, tumor suppressors, such as HIPK2, Ink4a/Arf, and APC, target CtBP1 degradation to induce apoptosis [1]. CtBP1 has many *in vitro* activities that are potentially oncogenic *in vivo*, including inhibition of apoptosis and promotion of epithelial-mesenchymal transition (EMT) [3,6]. Although CtBP1 potentially antagonizes

*Send correspondence to: Xiao-Jing Wang, MD, PhD, Department of Pathology, University of Colorado, Denver, Aurora, CO 80045, (303)-724-3001, Fax: (303)-724-4730, xj.wang@UCDenver.edu. Qinghong Zhang, PhD, Department of Dermatology, University of Colorado, Denver, Aurora, CO 80045, (303)-724-4051, Fax: (303)-724-4048, Qinghong.Zhang@UCDenver.edu.

#Co-first author

Conflict of Interests

The authors declare no conflict of interests.

multiple tumor suppressors at the molecular level *in vitro* [1], its targets are context-specific. In this study, we examined the expression of this co-repressor in human breast cancer and explored its potential contribution to tumorigenic pathways in breast cancer.

E-cadherin is a cell–cell adhesion protein with a prominent role in epithelial differentiation. Data from model systems suggest E-cadherin is a potent invasion/tumor suppressor of breast cancer [7] [8,9] [10]. Consistent with this role in breast cancer progression, partial or complete loss of E-cadherin expression has been found to correlate with poor prognosis in breast cancer patients [11] [12–14]. Another important tumor suppressor in breast cancer, *Brcal*, plays a central role in DNA damage repair, maintaining genomic stability. Individuals with germline mutations in *Brcal* carry an 80% lifetime risk of developing breast cancer [15,16]. Approximately 40% of non-inherited sporadic forms of breast cancer demonstrate a deficiency in *Brcal* expression [17], suggesting a large percentage of these tumors are associated with altered transcriptional/translational regulation of this tumor suppressor. Previously, we found that CtBP1 can repress the *E-cadherin* gene in lung cancer cells [18]. Our recent study demonstrated *Brcal* was under transcriptional control of CtBP1 in HNSCC [19]. Therefore, we studied the transcriptional regulation of *E-cadherin* and *Brcal* gene by CtBP1 during breast cancer progression.

Material and Methods

Consecutive slides of human breast cancer array BR1502 and normal breast tissue array BRN801 were purchased from US Biomax. The anti-CtBP1 antibody (Millipore) was raised against the 16 C-terminal amino acids of CtBP1 that are unique for CtBP1. To assess the correlation between CtBP1 and the tumor suppressors E-cadherin and *Brcal*, antibodies against E-cadherin (BD Bioscience), *Brcal* (Santa Cruz), and CtBP1 (Millipore) were used to stain consecutive tissue sections as we previously described [20]. Evaluation of CtBP1, *Brcal*, and E-cadherin staining of human tissue samples was performed by 2 independent investigators using previously described methods [20].

Breast cancer MCF7 and MDA-MB-231 cells were maintained in DMEM with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO₂. ChIP assays were performed using an anti-CtBP1 antibody as described previously [18]. Primer sets encompassing *E-cadherin* and *Brcal* promoters were used to q-PCR-amplify ChIP samples. Cells were transfected using Lipofectamine 2000 in suspension, with 100 nM scrambled siRNA (control) or siRNA targeting CtBP1 (siCtBP1) [6]. Cells were immediately transferred to 0.5 ml DMEM with FBS in 24 well plates and incubated at 37°C for 48 h. E-cadherin expression was detected by immunofluorescence staining using an E-cadherin antibody (BD Bioscience) [21]. Mitomycin C-induced DNA repair foci formation was assayed using a *Brcal* antibody (Santa Cruz) as we previously described [20]. Total RNA was isolated using TRIzol (Invitrogen) and qRT-PCR was performed as previously described [18]. An 18S probe was used as an internal control. The relative RNA expression levels were determined by normalizing to internal controls; values were calculated using the comparative Ct method. Samples were assayed in triplicate for each experiment and at least two independent experiments were performed. Data are presented as mean ± SEM from a representative experiment.

Results

CtBP1 was initially recognized as an adenoviral E1A-binding protein and its over-activation, in combination with a mutant Ras, leads to tumorigenesis and metastasis, suggesting CtBP1 plays a critical role in oncogenesis [22] [23] [24]. The underlying molecular mechanisms of CtBP1 in oncogenesis could be linked to its function as a

transcriptional co-repressor of tumor suppressors, including E-cadherin, p16INK4a, and p15INK4b [1]. To assess the potential involvement of CtBP1 in breast cancer development, we stained a human breast cancer array with an anti-CtBP1 antibody. Positive nuclear CtBP1 staining was found in 92% of invasive ductal breast cancer cases (BR1502, Biomax) (Fig. 1, right panels); in contrast, only 4% of normal breast tissue (BRN801, Biomax) stained positive for CtBP1 (Fig. 1, left panels).

Because positive CtBP1 staining was detected in human breast cancer cells, we examined if CtBP1 can repress the tumor suppressor *Brca1*. CtBP1 serves a key role in cellular regulation by binding to a variety of transcriptional repressors critical for development and tumorigenesis [25]. The expression of these transcription repressors might be cell- or tissue-specific, resulting in different responses to CtBP1-mediated repression. CtBP1 potentially interacts with many components assembled at the *Brca1* promoter including direct association with DNA binding proteins or indirect interactions with co-repressor complexes containing the CtBP1 binding adaptor protein, CtIP [1,26]. Therefore, we assessed if CtBP1 was recruited to the *Brca1* gene to repress transcription in breast cancer cells. We performed chromatin immunoprecipitation (ChIP) to identify CtBP1 binding sites in the *Brca1* promoter region in MDA-MB-231 cells (Fig. 2A). A CtBP1 binding site was found surrounding the transcriptional start site of the *Brca1* promoter, similar to the CtBP1 binding site in head and neck squamous cell carcinoma (HNSCC) cells [19]. Our previous study demonstrated this CtBP1 binding site confers transcriptional repression to the *Brca1* gene [19]. *Brca1* mRNA levels increased 3 fold when CtBP1 was abrogated in MDA-MB-231 cells (Fig. 2B). Accordingly, Brca1 protein was upregulated when CtBP1 was knocked down in MDA-MB-231 cells (Fig. 2C).

To further assess if restoration of *Brca1* expression by CtBP1 knockdown confers rescue of Brca1 function, we examined Brca1-mediated DNA repair foci formation by immunofluorescence staining in MDA-MB-231 cells and MDA-MB-231-siCtBP1 cells treated with mitomycin C (MMC). Under normal conditions, Brca1 translocates to sites of MMC-induced DNA damage with other members of the Fanc/Brca pathway to form DNA repair nuclear foci [27]. 24 h after 10 ng/ml MMC treatment, only about 10% of MDA-MB-231 cells were able to form Brca1 foci, whereas 32% of MDA-MB-231 cells with siCtBP1 48 h knockdown were able to form MMC-induced DNA repair foci (Fig. 2D). These data show that CtBP1-mediated *Brca1* repression abrogates Brca1 function.

Our study suggests that under normal conditions, Brca1 expression in breast cells is not repressed by low levels of CtBP1. However, during breast cancer carcinogenesis, increased levels of CtBP1 induce repression of *Brca1*. To determine the relative expression of Brca1 and CtBP1 in breast cancer, we performed IHC for CtBP1 and Brca1 on invasive ductal breast cancer cases using serial slides of a breast cancer array (BR1502, Biomax). Brca1 loss was detected in 62% (34/55) of the CtBP1-positive invasive ductal breast cancer cases; 0% (0/5) of the Brca1 loss cases with CtBP1-negative staining, suggesting an inverse correlation between Brca1 and CtBP1 in these lesions (Fig. 3 and Table 1). These data strongly support CtBP1 repression of *Brca1* expression *in vivo*; therefore CtBP1 might serve as a biomarker, alone or in combination with Brca1 loss, for breast cancer development.

The ability of tumors to metastasize is a hallmark of malignancy. A critical event during metastasis is adhesion reduction, facilitating tumor cell invasion into surrounding tissues and vascular channels, ultimately leading to cancer progression. E-cadherin-mediated cell-cell adhesion is essential for maintaining homeostasis and architecture of epithelial tissues. Down-regulation of E-cadherin expression occurs concomitantly with dedifferentiation and invasion of epithelial cells during tumorigenesis [8,9]. Consequently, E-cadherin and its associated complex are thought to be key mediators of tumor cell invasion [28]. To explore

functional consequences of CtBP1 up-regulation in breast cancer, we assessed if the *E-cadherin* gene is suppressed by CtBP1 in breast cancer cells.

We performed a ChIP assay using the anti-CtBP1 antibody to identify CtBP1 binding sites in the *E-cadherin* promoter region of MDA-MB-231 cells (Fig. 4A). Compared to the normal IgG, the anti-CtBP1 antibody pulled down 2–3 fold more *E-cadherin* promoter, suggesting CtBP1 was recruited to the *E-cadherin* promoter. Furthermore, *E-cadherin* mRNA levels increased 6 fold when CtBP1 was abrogated in MDA-MB-231 cells (Fig. 4B), demonstrating CtBP1 transcriptionally represses *E-cadherin* in human breast cancer cells. *E-cadherin* protein was increased by CtBP1 knockdown as well (Fig. 4C). A similar effect has been observed in MCF7 cells [29].

Next, we examined *E-cadherin* expression by immunofluorescence staining using MDA-MB-231 and MDA-MB-231-siCtBP1 cells. Weak *E-cadherin* staining was observed in a punctate pattern along the cell edge in MDA-MB-231 cells, whereas MDA-MB-231 cells with siCtBP1 48 h knockdown for exhibited stronger *E-cadherin* staining (Fig. 4D). These data show CtBP1-mediated *E-cadherin* transcriptional repression abrogates *E-cadherin* expression in breast cancer cells, suggesting CtBP1 might contribute to metastatic potential during breast cancer development.

To explore the potential regulation of *E-cadherin* by CtBP1 in breast cancer, we performed IHC for CtBP1 and *E-cadherin* using serial slides of a breast cancer array. *E-cadherin* loss was detected in 83% (45/57) of the invasive ductal breast cancer cases with CtBP1-positive staining; whereas *E-cadherin* loss was not detected in the malignant breast cancer cases lacking CtBP1 staining (0/5), suggesting an inverse correlation between *E-cadherin* and CtBP1 in these lesions as well (Fig. 5 and Table 2). These data strongly support CtBP1 repression of *E-cadherin* expression *in vivo*.

Discussion

Breast cancer is the most frequently occurring cancer in women, accounting for over 20% of all cases. Ever since the discovery of CtBP1, several attempts were made to assess the involvement of CtBP1 in cancer development, but they failed due to the lack of antibodies specific for CtBP1 IHC staining. The CtBP1 specific antibody used here provides a useful tool for exploring CtBP1 expression in human cancer. For the first time, we found CtBP1-positive staining in 92% of invasive ductal breast cancer cases, in comparison to 4% CtBP1-positive staining in normal breast tissue. CtBP1 has been functionally linked to DNA damage repair and EMT. In this study we confirmed transcriptional regulation of *Brca1* and *E-cadherin* by CtBP1 in breast cancer cells and identified a close correlation between CtBP1 expression and loss of *Brca1* and *E-cadherin* in malignant breast cancer samples. Consistent with our findings, CtBP1 repression of *Brca1* and *E-cadherin* has also been reported in breast cancer MCF7 cells [29,30]. All these lines of evidence support the notion that CtBP1 has a critical role in the development of breast cancer.

Studies during the past decade have demonstrated CtBP1 plays a central role in multiple pathways contributing to tumorigenesis. For example, CtBP1 is known to suppress *E-cadherin*, a gatekeeper for tumor invasion and metastasis [3,4]. CtBP1's newly identified role suppressing *Brca1* adds another dimension: CtBP1 might promote genomic instability [19] [30]. Identified as a breast cancer susceptibility gene, individuals harboring mutations in *Brca1* carry an 80% lifetime risk of developing breast cancer [15,16]. *Brca1* loss is found in a higher percentage of sporadic breast cancers, even though very few cases are associated with mutation in *Brca1* [17], suggesting additional regulation of *Brca1* expression at either the transcriptional or post-transcriptional level. *E-cadherin* is a potent invasion/tumor suppressor of breast cancer that is frequently down-regulated during metastasis. Partial or

complete loss of E-cadherin expression correlates with poor prognosis in breast cancer patients [11] [31] [13] [14]. The *E-cadherin* gene is located on human chromosome 16q22.1 [32], a region frequently affected with loss of heterozygosity in sporadic breast cancer [31]. Invasive lobular breast carcinomas, which are typically E-cadherin-negative, often show inactivating mutations in combination with loss of heterozygosity of the wild-type *E-cadherin* allele. In contrast, most ductal breast cancers show heterogeneous loss of E-cadherin expression, associated with epigenetic transcriptional down-regulation. The inverse correlation between *Brcal* and E-cadherin levels with the expression of CtBP1 in human breast cancer samples indicate CtBP1 functions as a master regulator of key mediators in breast cancer development, therefore, CtBP1 might serve as a potential biomarker.

Of note, CtBP1-positive staining was detected in breast cancer tissue at a higher percentage than the loss of *Brcal* and E-cadherin protein, having higher correlation with E-cadherin loss than with *Brcal* loss. As a transcriptional co-repressor, CtBP1 must be recruited to DNA-binding transcriptional repressors to carry out its function [25]. The status of respective transcriptional repressor(s) might vary in breast cancer tissue and affect *Brcal* expression. In fact, *Brcal* has been shown to be regulated by a variety of transcription factors and co-factors, which link *Brcal* transcription to distinct signal transduction events [33–39]. Nonetheless, the inverse correlation of *Brcal* and E-cadherin with CtBP1 in breast cancer samples suggests CtBP1 contributes to breast cancer development by suppressing transcription of genes governing DNA damage repair, genomic stability and tumor progression.

Highly aggressive, rapidly growing tumors can become hypoxic, leading to high NADH levels [40]. We have shown the transcriptional co-repressor CtBP has the unique ability to sense elevated levels of free nuclear NADH during tumor progression and transmit this information to complexes that regulate gene expression, including E-cadherin and *Brcal* [41] [19]. Thus, the redox-sensing property of CtBP provides a regulatory switch for the expression of these tumor suppressive genes under hypoxic conditions, setting tumors on the road to metastasis [42]. Conversely, NADH downregulation blocked CtBP repression of *Brcal* [30]. Since E-cadherin and *Brcal* are common tumor suppressors in multiple organs/tissues, it will be interesting to assess if CtBP1-mediated transcriptional repression contributes to the growth of multiple cancer types. Whether CtBP1 expression is of prognostic value awaits further investigation.

Acknowledgments

This work was supported by grants from the NIH, R01DE15953 (to X. J. W.) and R01CA115468 (to Q.Z.).

References

1. Chinnadurai G. The transcriptional corepressor CtBP: a foe of multiple tumor suppressors. *Cancer Res.* 2009; 69(3):731–734. [PubMed: 19155295]
2. Furusawa T, Moribe H, Kondoh H, Higashi Y. Identification of CtBP1 and CtBP2 as corepressors of zinc finger-homeodomain factor deltaEF1. *Mol Cell Biol.* 1999; 19(12):8581–8590. [PubMed: 10567582]
3. Grooteclaes M, Deveraux Q, Hildebrand J, Zhang Q, Goodman RH, Frisch SM. C-terminal-binding protein corepresses epithelial and proapoptotic gene expression programs. *Proc Natl Acad Sci U S A.* 2003; 100(8):4568–4573. [PubMed: 12676992]
4. Grooteclaes ML, Frisch SM. Evidence for a function of CtBP in epithelial gene regulation and anoikis. *Oncogene.* 2000; 19(33):3823–3828. [PubMed: 10949939]
5. Mroz EA, Baird AH, Michaud WA, Rocco JW. COOH-terminal binding protein regulates expression of the p16INK4A tumor suppressor and senescence in primary human cells. *Cancer Res.* 2008; 68(15):6049–6053. [PubMed: 18676825]

6. Zhang Q, Yoshimatsu Y, Hildebrand J, Frisch SM, Goodman RH. Homeodomain interacting protein kinase 2 promotes apoptosis by downregulating the transcriptional corepressor CtBP. *Cell*. 2003; 115(2):177–186. [PubMed: 14567915]
7. Frixen UH, Behrens J, Sachs M, et al. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol*. 1991; 113(1):173–185. [PubMed: 2007622]
8. Meiners S, Brinkmann V, Naundorf H, Birchmeier W. Role of morphogenetic factors in metastasis of mammary carcinoma cells. *Oncogene*. 1998; 16(1):9–20. [PubMed: 9467938]
9. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature*. 1998; 392(6672):190–193. [PubMed: 9515965]
10. Vlemminckx K, Vakaet L Jr, Mareel M, Fiers W, van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell*. 1991; 66(1):107–119. [PubMed: 2070412]
11. Heimann R, Lan F, McBride R, Hellman S. Separating favorable from unfavorable prognostic markers in breast cancer: the role of E-cadherin. *Cancer Res*. 2000; 60(2):298–304. [PubMed: 10667580]
12. Hunt NC, Douglas-Jones AG, Jasani B, Morgan JM, Pignatelli M. Loss of E-cadherin expression associated with lymph node metastases in small breast carcinomas. *Virchows Arch*. 1997; 430(4):285–289. [PubMed: 9134039]
13. Oka H, Shiozaki H, Kobayashi K, et al. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res*. 1993; 53(7):1696–1701. [PubMed: 8453644]
14. Siitonen SM, Kononen JT, Helin HJ, Rantala IS, Holli KA, Isola JJ. Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer. *Am J Clin Pathol*. 1996; 105(4):394–402. [PubMed: 8604681]
15. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. *Lancet*. 1994; 343(8899):692–695. [PubMed: 7907678]
16. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet*. 1998; 62(3):676–689. [PubMed: 9497246]
17. Turner NC, Reis-Filho JS, Russell AM, et al. BRCA1 dysfunction in sporadic basal-like breast cancer. *Oncogene*. 2007; 26(14):2126–2132. [PubMed: 17016441]
18. Zhang Q, Wang SY, Nottke AC, Rocheleau JV, Piston DW, Goodman RH. Redox sensor CtBP mediates hypoxia-induced tumor cell migration. *Proc Natl Acad Sci U S A*. 2006; 103(24):9029–9033. [PubMed: 16740659]
19. Deng Y, Liu J, Han G, et al. Redox-dependent Brca1 transcriptional regulation by an NADH-sensor CtBP1. *Oncogene*. 2010; 29(50):6603–6608. [PubMed: 20818429]
20. Bornstein S, White R, Malkoski S, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. *J Clin Invest*. 2009; 119(11):3408–3419. [PubMed: 19841536]
21. Hoot KE, Lighthall J, Han G, et al. Keratinocyte-specific Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. *J Clin Invest*. 2008; 118(8):2722–2732. [PubMed: 18618014]
22. Boyd JM, Subramanian T, Schaeper U, La Regina M, Bayley S, Chinnadurai G. A region in the C-terminus of adenovirus 2/5 E1a protein is required for association with a cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis. *Embo J*. 1993; 12(2):469–478. [PubMed: 8440238]
23. Subramanian T, La Regina M, Chinnadurai G. Enhanced ras oncogene mediated cell transformation and tumorigenesis by adenovirus 2 mutants lacking the C-terminal region of E1a protein. *Oncogene*. 1989; 4(4):415–420. [PubMed: 2524023]
24. Schaeper U, Boyd JM, Verma S, Uhlmann E, Subramanian T, Chinnadurai G. Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation. *Proc Natl Acad Sci U S A*. 1995; 92(23):10467–10471. [PubMed: 7479821]

25. Chinnadurai G. CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Mol Cell*. 2002; 9(2):213–224. [PubMed: 11864595]
26. Chinnadurai G. CtIP, a candidate tumor susceptibility gene is a team player with luminaries. *Biochim Biophys Acta*. 2006; 1765(1):67–73. [PubMed: 16249056]
27. D'Andrea AD, Grompe M. The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer*. 2003; 3(1):23–34. [PubMed: 12509764]
28. Beavon IR. The E-cadherin-catenin complex in tumour metastasis: structure, function and regulation. *Eur J Cancer*. 2000; 36(13 Spec):1607–1620. [PubMed: 10959047]
29. Lee S, Hong S, Kim S, Kang S. Ataxin-1 occupies the promoter region of E-cadherin in vivo and activates CtBP2-repressed promoter. *Biochim Biophys Acta*. 2011
30. Di LJ, Fernandez AG, De Siervi A, Longo DL, Gardner K. Transcriptional regulation of BRCA1 expression by a metabolic switch. *Nat Struct Mol Biol*. 2010; 17(12):1406–1413. [PubMed: 21102443]
31. Berx G, Van Roy F. The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. *Breast Cancer Res*. 2001; 3(5):289–293. [PubMed: 11597316]
32. Berx G, Cleton-Jansen AM, Nollet F, et al. E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. *Embo J*. 1995; 14(24):6107–6115. [PubMed: 8557030]
33. Atlas E, Stramwasser M, Mueller CR. A CREB site in the BRCA1 proximal promoter acts as a constitutive transcriptional element. *Oncogene*. 2001; 20(48):7110–7114. [PubMed: 11704836]
34. Baker KM, Wei G, Schaffner AE, Ostrowski MC. Ets-2 and components of mammalian SWI/SNF form a repressor complex that negatively regulates the BRCA1 promoter. *J Biol Chem*. 2003; 278(20):17876–17884. [PubMed: 12637547]
35. Bindra RS, Gibson SL, Meng A, et al. Hypoxia-induced down-regulation of BRCA1 expression by E2Fs. *Cancer Res*. 2005; 65(24):11597–11604. [PubMed: 16357170]
36. Bindra RS, Glazer PM. Basal repression of BRCA1 by multiple E2Fs and pocket proteins at adjacent E2F sites. *Cancer Biol Ther*. 2006; 5(10):1400–1407. [PubMed: 17106239]
37. De Siervi A, De Luca P, Byun JS, et al. Transcriptional autoregulation by BRCA1. *Cancer Res*. 2010; 70(2):532–542. [PubMed: 20068145]
38. Mueller CR, Roskelley CD. Regulation of BRCA1 expression and its relationship to sporadic breast cancer. *Breast Cancer Res*. 2003; 5(1):45–52. [PubMed: 12559046]
39. Thakur S, Croce CM. Positive regulation of the BRCA1 promoter. *J Biol Chem*. 1999; 274(13):8837–8843. [PubMed: 10085126]
40. Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst*. 2001; 93(4):266–276. [PubMed: 11181773]
41. Zhang Q, Piston DW, Goodman RH. Regulation of corepressor function by nuclear NADH. *Science*. 2002; 295(5561):1895–1897. [PubMed: 11847309]
42. Beavon IR. Regulation of E-cadherin: does hypoxia initiate the metastatic cascade? *Mol Pathol*. 1999; 52(4):179–188. [PubMed: 10694937]

Figure 1. CtBP1 positive staining in invasive ductal breast cancers. Normal breast tissue array US Biomax BRN801 (left) and human malignant breast tissue array US Biomax BR1502 (right) were stained for CtBP1. Scale bar = 40 μ m.

Figure 2.

CtBP1 represses *Brca1* expression. A. CtBP1 binding to the *Brca1* regulatory element. Human breast cancer MDA-MB-231 cells were used for ChIP assay with an anti-CtBP1 antibody. Primers encompassing the *Brca1* promoter were used to q-PCR-amplify the ChIP sample. * $p < 0.05$ vs. IgG. B. CtBP1 knockdown in MDA-MB-231 cells increased *Brca1* mRNA; * $p < 0.05$ vs. control cells. MDA-MB-231 cells were transfected with scrambled siRNA (Control) or siRNA targeting CtBP1 (siCtBP1) [6] and incubated at 37°C for 48 h. The relative RNA expression levels were determined by normalizing with internal controls, values were calculated using the comparative Ct method. Samples were assayed in triplicate for each experiment and at least two independent experiments were performed. Data are presented as mean $\pm$ SEM from a representative experiment. Inset shows the knock down of CtBP1 in MDA-MB-231 cells. C. CtBP1 knockdown in MDA-MB-231 cells increased *Brca1* protein. Tubulin serves as a loading control. D. CtBP1 knockdown increases MMC-induced DNA repair foci formation. MDA-MB-231 cells were transfected with scrambled siRNA (control) or siRNA targeting CtBP1 (siCtBP1) for 48 hr. Anti-*Brca1* antibody was used to stain DNA repair foci 24 h after 10 ng/ml MMC treatment. Scale bar = 5 μ m.

Brca1**CtBP1** **Figure 3.**

Correlation between CtBP1 up-regulation and Brca1 down-regulation in invasive ductal breast cancers. Immunohistochemical staining was performed using antibodies against Brca1 and CtBP1 to stain consecutive tissue sections as we previously described [20]. Evaluation of CtBP1 and Brca1 staining of human invasive ductal breast cancer samples was performed by 2 independent investigators using methods described previously [20]. Note a lesion with CtBP1 nuclear staining (D) showed negative staining for Brca1 in the consecutive section (C). In contrast, a lesion with CtBP1 staining in the cytoplasm but little in the nucleus (B) exhibited positive Brca1 staining (A). Scale bar = 20µm.

Figure 4.

CtBP1 represses *E-cadherin* expression. A. CtBP1 binding to the *E-cadherin* regulatory element. MDA-MB-231 cells were used for ChIP assay with an anti-CtBP1 antibody. Primers encompassing the *E-cadherin* promoter were used to q-PCR-amplify the ChIP sample. * $p < 0.05$ vs. IgG. B. CtBP1 knockdown in MDA-MB-231 cells increased *E-cadherin* mRNA; * $p < 0.05$ vs. control cells. MDA-MB-231 cells were transfected with scrambled siRNA (Control) or siRNA targeting CtBP1 (siCtBP1) [6] and incubated at 37°C for 48 h. Total RNA was isolated and qRT-PCR was performed as previously described [18]. An 18S probe was used as an internal control. Data are presented as mean $\pm$ SEM from a representative experiment. C. CtBP1 knockdown in MDA-MB-231 cells increased *E-cadherin* protein. Tubulin serves as a loading control. D. CtBP1 knockdown increases *E-cadherin* expression in MDA-MB-231. Anti *E-cadherin* was used to stain cells without (SC) or with siCtBP1 treatment (siCtBP1). Scale bar = 15μm.

E-cadherin**CtBP1**

Figure 5. Correlation between CtBP1 up-regulation and E-cadherin down-regulation in invasive ductal breast cancers. Immunohistochemical staining was performed using antibodies against E-cadherin and CtBP1 to stain consecutive tissue sections as we previously described [20]. Note a lesion without CtBP1 staining (B) exhibited positive E-cadherin staining (A). In contrast, a lesion with CtBP1 nuclear staining (D) showed negative staining for E-cadherin in the consecutive section (C). Scale bar = 40µm.

Table 1

Correlation between CtBP1 positive staining and Brca1 down-regulation in invasive ductal breast cancers.

	Number of Cases/Total Cases	
% of CtBP1 positive (+)	55/60 (92%)	
	CtBP1(+)	CtBP1(−)
% of Brca1 Down-regulation in CtBP1 (+) or (−) cases	34/55 (62%)*	0/5 (0%)

*
p<0.05 between CtBP1 (+) and CtBP1 (−) groups.

Table 2

Correlation between CtBP1 positive staining and E-cadherin down-regulation in invasive ductal breast cancers.

	Number of Cases/Total Cases	
% of CtBP1 positive (+)	57/62 (92%)	
	CtBP1(+)	CtBP1(-)
% of E-cadherin Down-regulation in CtBP1 (+) or (-)cases	47/57 (82%) [#]	0/5 (0%)

[#] p<0.01 between CtBP1 (+) and CtBP1 (-) groups.