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Molecular Cancer Therapeutics

Evaluation of CDK12 Protein Expression as a Potential Novel Biomarker for DNA Damage Response–Targeted Therapies in Breast Cancer

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Abstract

Disruption of Cyclin-Dependent Kinase 12 (*CDK12*) is known to lead to defects in DNA repair and sensitivity to platinum salts and PARP1/2 inhibitors. However, *CDK12* has also been proposed as an oncogene in breast cancer. We therefore aimed to assess the frequency and distribution of CDK12 protein expression by IHC in independent cohorts of breast cancer and correlate this with outcome and genomic status. We found that 21% of primary unselected breast cancers were CDK12 high, and 10.5% were absent, by IHC. CDK12 positivity correlated with HER2 positivity but was not an independent predictor of breast cancer–specific survival taking HER2 status into account; however, absent CDK12 protein expression significantly correlated with a triple-negative phenotype. Interestingly, CDK12 protein absence was associated with reduced expression of a number of DDR proteins including ATR, Ku70/Ku80, PARP1, DNA-PK, and γH2AX, suggesting a novel mechanism of CDK12-associated DDR dysregulation in breast cancer. Our data suggest that diagnostic IHC quantification of CDK12 in breast cancer is feasible, with CDK12 absence possibly signifying defective DDR function. This may have important therapeutic implications, particularly for triple-negative breast cancers. *Mol Cancer Ther; 17(1); 306–15. ©2017 AACR.*

Introduction

Breast cancer is a complex disease comprising a variety of molecular and clinically distinct subtypes. Substantial progress has been made in the management of breast cancer mortality over the last 25 years, in part due to improved treatment modalities such as endocrine therapies, HER2-targeted therapy, and combination chemotherapies (1–4). However, a proportion of sporadic primary breast cancer remain difficult to treat. Hence, there is an urgent need for stratification and biomarker discovery within this cohort.

The CycK/CDK12 (Cyclin K/Cyclin dependent kinase 12) complex is involved in the regulation of RNA polymerase II (RNA pol II) and mRNA processing (5–7) and is known to protect normal cells from genomic instability by regulating the transcription of DNA damage response (DDR) genes (8). Moreover, CDK12 has been postulated as a tumor suppressor gene in high-grade serous ovarian cancer (HGSOC), where it is one of the only significantly recurrently mutated genes (9). Recurrent point mutations have been shown to abrogate the functional activity of CDK12, resulting in defects in multiple DNA repair pathways, leading to genomic instability, downregulation of some homologous recombination (HR) genes such as *BRCA1*, *FANCI*, or *FANCD2* (10, 11) and selective sensitivity to both platinum agents and PARP1/2 inhibitors (12, 13). Indeed, recent data in HGSOC suggests that CDK12-inactivated tumors have a unique signature of genomic instability characterized by frequent mega-sized gains scattered over the genome, which is a result of numerous tandem duplications, indicative of gross defects in DNA repair (14). In addition, recent profiling studies have also identified *CDK12* mutations in primary and castration-resistant prostate cancer that are mutually exclusive with other mutations in DNA repair genes (15, 16), and akin to HGSOC, result in large tandem duplications (14). On the other hand, in breast cancer, *CDK12* gene amplification often co-occurs with *ERBB2* amplification as both are colocated at locus Ch17q12 (17, 18), and CDK12 overexpression has been correlated with indicators of aggressive disease,

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suggesting that CDK12 could act as a oncogenic driver and prognostic biomarker in breast cancer as a result of this colocation (19).

We have previously shown that in breast cancer, *CDK12* is recurrently targeted by both DNA rearrangements (13% of HER2-amplified breast cancer) and recurrent point mutations (2.6% of unselected breast cancer; ref. 13) in a similar manner to HGSOC, and that loss of CDK12 in breast cancer models confers sensitivity to PARP1/2 inhibitors *in vitro* through defects in HR (12, 13). Loss of CDK12 in breast cancer may therefore signify response to platinum salts and/or PARP1/2 inhibitors (12, 13).

Here we sought to (i) investigate the distribution and frequency of CDK12 protein expression in a large series of unselected and Herceptin-treated HER2-positive breast cancer, using IHC and examine any correlation with survival; (ii) evaluate CDK12 protein and mRNA expression with genomic alterations and (iii) assess whether CDK12 would constitute an oncogenic driver in *CDK12*-amplified tumors.

Materials and Methods

Tissue microarray patient cohorts

Unselected breast cancer. Primary operable breast cancer cases ($n =$ 1,650) from the Nottingham Tenovus Primary Breast Carcinoma Series were utilized as described previously (20–22). Patients were under the age of 71 years (median, 55 years), diagnosed between 1986 and 1999, and treated uniformly in a single institution. Clinicopathologic parameters for this series are summarized in Supplementary Table S1.

HER2-positive adjuvant trastuzumab series. The HER2-positive adjuvant trastuzumab series comprises 143 primary operable breast cancer from patients presenting between 2003 and 2010 who received adjuvant trastuzumab (21). HER2 status was determined according to the American Society of Clinical Oncology (ASCO) guidelines as described previously (21). Clinicopathologic parameters for this series are summarized in Supplementary Table S2.

METABRIC Nottingham breast cancers. This series comprised 282 primary breast cancers from Nottingham, which form part of the METABRIC cohort (ref. 23; Supplementary Table S3).

Tissue microarray construction. Tumor samples were arrayed as described previously (22). Briefly, one core per tumor of 0.6-mm thickness was obtained from the most representative areas then reembedded in microarray blocks.

CDK12 IHC. IHC was optimized in-house, using a standard labeled polymer technique, on 4-μm sections of formalin-fixed paraffin embedded (FFPE) normal human tonsil; cell blocks containing the MCF7 breast cancer cell line known to express CDK12 transfected with a previously validated siRNA pool targeting CDK12 or nontargeting control and BT474 cells as a positive control (13) (Fig. 1). Cells were cultured as described previously (13) and authenticated by short tandem repeat (STR) typing using the StemElite Kit (Promega). Briefly, slides were dewaxed in xylene and rehydrated through graded alcohols. Following heat-induced antigen retrieval in citrate buffer (pH 6.0), sections were incubated with a mouse anti-human CDK12 mAb (1:5,000 final dilution, Abcam clone 57311 that was raised against an immunogen peptide corresponding to amino acids 1,281–1,380 of Human CDK12) for one hour at room temperature. The staining was visualized using the Dako Flex Envision K8002 Kit (Dako), counterstained with Gill's hematoxylin (Leica). Sections were then dehydrated and mounted.

TMAs were assessed for nuclear CDK12 protein expression in the malignant epithelium only, using a modified Allred score (14). Only technically sound cores containing >20% invasive tumor cells were included in the analysis. Cores were evaluated for both intensity (0 = no stain; 1 = mild; 2 = moderate; 3 = strong), and percentage of epithelial cells that stained positive (0 = absent; 1 = background; 2 = 1%–25%; 3 = 26%–50%; 4 = 51%–75%; 5 = >75%; Fig. 1). Scores were derived from a sum of the intensity and percentage of immunoreactive cells; an average score of 0 for each tumor was considered negative/absent; a score of 7 or 8, high; and a score of 2–6 as intermediate expression. Scores of 1 were excluded from further analysis as these equated to background nonspecific staining. IHC staining and dichotomization of the other biomarkers included in this study were as per previous publications (24–30). Scoring was performed blinded to the study endpoint.

Mining of public datasets. To corroborate our findings, we reanalyzed publicly available data from The Cancer Genome Atlas (31, 32) and METABRIC (23) datasets, to ascertain the frequency of *CDK12* copy number breakpoints, somatic mutations, and methylation and correlate these with RNA expression levels. Low and high CDK12 gene expression were defined by using an optimal threshold for dichotomizing gene expression data as described previously (33). This was carried out by a stepwise analysis from 40 to 60 percentiles at an interval of 5. The cutoffs that displayed the highest prognostic significance with log-rank test were selected. In addition, analysis of published whole genome shRNA (34) and kinome-wide siRNA (35) genetic perturbation screens was performed to correlate cell viability of breast cancer cell lines with and without *CDK12* amplification after CDK12 knockdown.

Assessment of tandem duplicator phenotype. Affymetrix SNP6.0 copy number data of 224 METABRIC samples were preprocessed using PennCNV-affy package (affy: http://penncnv.openbioinformatics.org/en/latest/misc/credit/ and segmented absolute copy number and ploidy was established with ASCAT 2.1 (36). The two tandem duplication phenotypes were established as previously described in Watkins and colleagues, 2016 (37).

Statistical analysis

Retrospective statistical analysis was performed using SPSS 21.0 statistical software (SPSS Inc.), in compliance with reporting recommendations for tumor marker prognostic studies (REMARK) criteria (38). A χ^2or Fisher exact test of <0.05 was considered significant. Survival curves were analyzed by the method of Kaplan–Meier, with a P value <0.05 being considered significant with a 10-year breast cancer–specific survival as the endpoint. Multivariate survival analysis was carried out using CDK12 expression status, node status, estrogen and progesterone receptor (ER/PR) status, HER2 status, age, tumor size, and grade. A Student t test was employed to compare CDK12 expression of mined samples with genetic aberrations and normal controls. For comparisons, scores of 0 (absent) and high (7–8) were compared, given known correlations with high expression and amplification (19) and uncertainty regarding intermediate levels of expression.

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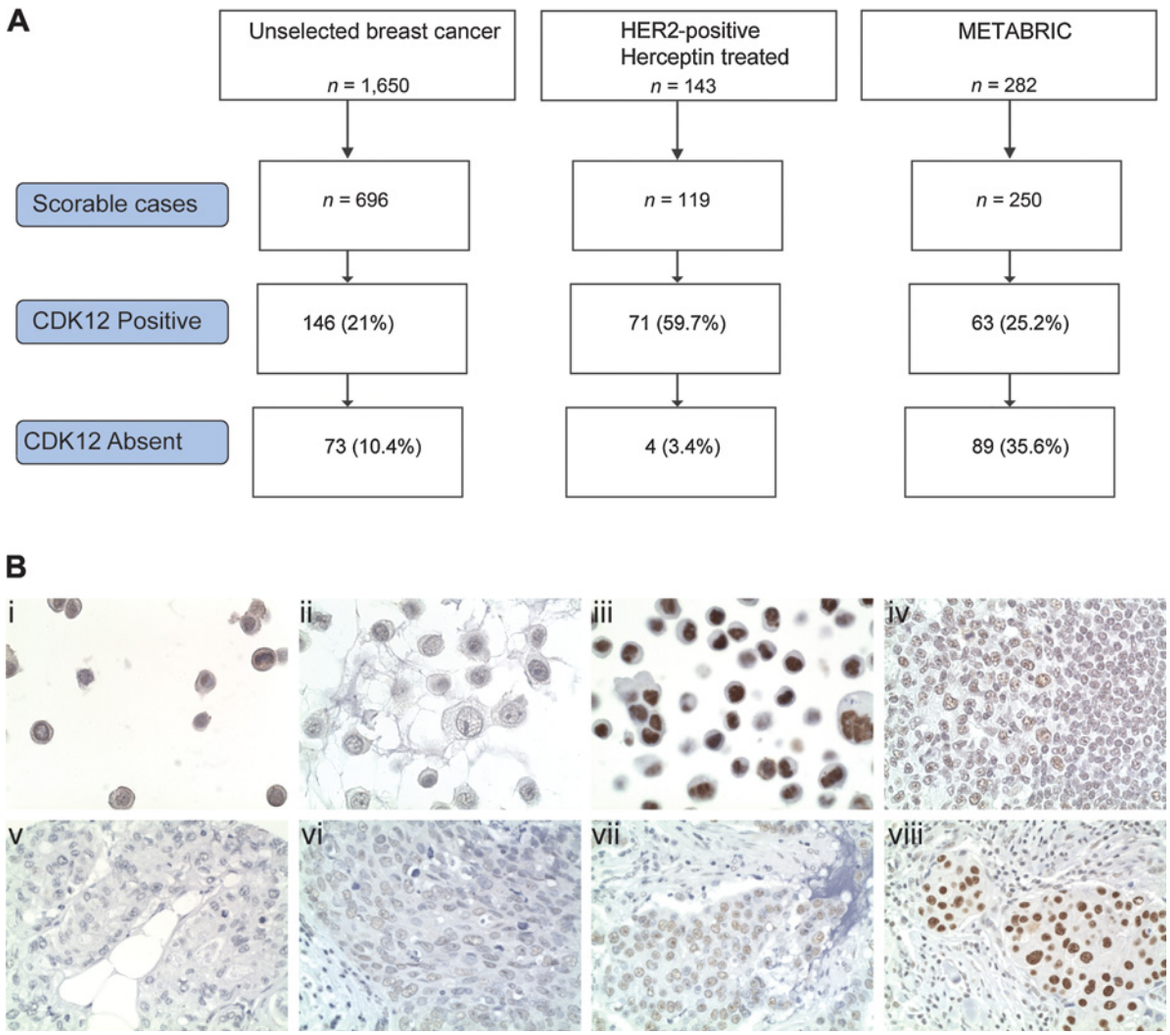

Figure 1.

Distribution of CDK12 protein expression in breast cancer. **A,** Modified CONSORT diagram depicting the distribution of CDK12-positive and -negative breast cancers in each of the cohorts analyzed. **B,** Representative micrographs of CDK12 protein expression in MCF7 cell line treated with nontargeting siRNA controls (**i**); MCF7 cell line treated with previously validated siRNA against CDK12 (ref. 13; **ii**); BT474 CDK12 amplified cells all at 400× magnification (**iii**); tonsil positive control (×200 magnification; **iv**). Representative images of staining intensity in primary breast cancers, where CDK12 expression was quantified using a modified Allred score, which assessed both intensity (highest score = 3) and percentage positivity (highest score = 5): (**v**) negative; (**vi**) 1+; (**vii**) 2+; and (**viii**) 3+; all images at 200× magnification. A score of 0 was considered absent and a score of 7 or 8, as high expression.

A $P < 0.05$ (two-sided) was considered statistically significant. Differential gene expression analysis of CDK12 absent versus high tumors with gene expression known to be involved in DNA repair was performed using data from METABRIC using Limma with FDR (false discovery rate) multiple correction adjustment.

Results

Distribution of CDK12 expression and clinicopathologic correlation

In the first instance, we assessed CDK12 protein expression by IHC in 696 unselected breast cancer samples that met the inclusion criteria (described in Methods; Fig. 1). Overall 73 of 696 tumors were absent/negative for CDK12 (10.5%) by IHC, and 146 had CDK12 high expression (21%; Fig. 1A; Supplementary Table S1). Breaking this down by subtype, in ER-positive patients, 50/510 (9.8%) and 101/510 (19.8%) were CDK12 absent and high; in HER2-positive patients, 3 of 102 (2.9%) and 55 of 102 (53.9%) were CDK12 absent and high; and in triple-negative patients, 21 of 123 (17%) and 17 of 123 (13.8%) were CDK12 absent and high, respectively. Expression of CDK12 significantly correlated with HER2 expression; 96% of CDK12-absent tumors were HER2 negative and 95% of HER2-positive tumors had high CDK12 expression ($P < 0.001$, χ^2 test). Interestingly, no significant correlation of CDK12 expression with ER or PR status was observed, but a greater proportion of CDK12-absent tumors showed a triple-

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negative phenotype (21/73, 29%) than CDK12-high tumors (17/143, 11.9%, $P = 0.002$, χ^2 test; Supplementary Table S1). There was no association with CDK12-high expression and breast cancer–specific survival in this cohort [$P = 0.354$; HR = 1.295; 95% CI = 0.75–2.24, log-rank (Mantel–Cox), Fig. 2A; Supplementary Table S4].

These findings were validated in a subset of tumors from the METABRIC cohort of unselected breast cancer, in which CDK12 was highly expressed in 63 of 250 tumors (25.2%) and absent in 89 of 250 tumors (35.6%; Fig. 1; Supplementary Table S3). Again, a significant correlation with HER2 status was observed, with 83 of 89 (93.3%) of CDK12-absent tumors being HER2 negative and 13 of 36 (36.1%) of HER2-amplified tumors being CDK12 high ($P < 0.0001$, χ^2 test). CDK12 expression also conferred a significantly poorer breast cancer–specific survival [$P < 0.001$; HR = 3.161; 95% CI = 1.632–6.125, log-rank (Mantel–Cox)] in this cohort in univariate analysis (Fig. 2B). This was also significant in multivariate comparisons when taking account for HER2 positivity ($P = 0.038$; HR = 2.26; 95% CI = 1.045–4.887; Supplementary Table S4). These associations were further corroborated at the mRNA level in a larger cohort of primary tumors ($n = 1{,}961$) from METABRIC, where high CDK12 expression was associated with a worse breast cancer–specific survival [$P < 0.001$; HR = 1.28; 95% CI = 1.17–1.41; log-rank (Mantel–Cox); Fig. 2C]; however, this was not substantiated in multivariate analysis when taking HER2 into account (Supplementary Table S4).

We previously identified a proportion of HER2-amplified tumors harboring out-of-frame *CDK12* fusion genes. As CDK12 is known to map to the smallest region within the *HER2* amplicon (17, 18), these fusions are the result of an amplification breakpoint in the *HER2* amplicon that converges on CDK12, disrupting its expression (13). We also found that tumor cells with loss of CDK12 due to the presence of a breakpoint in the *HER2* amplicon were sensitive to PARP1/2 inhibitors suggesting that a fraction of HER2-amplified patients

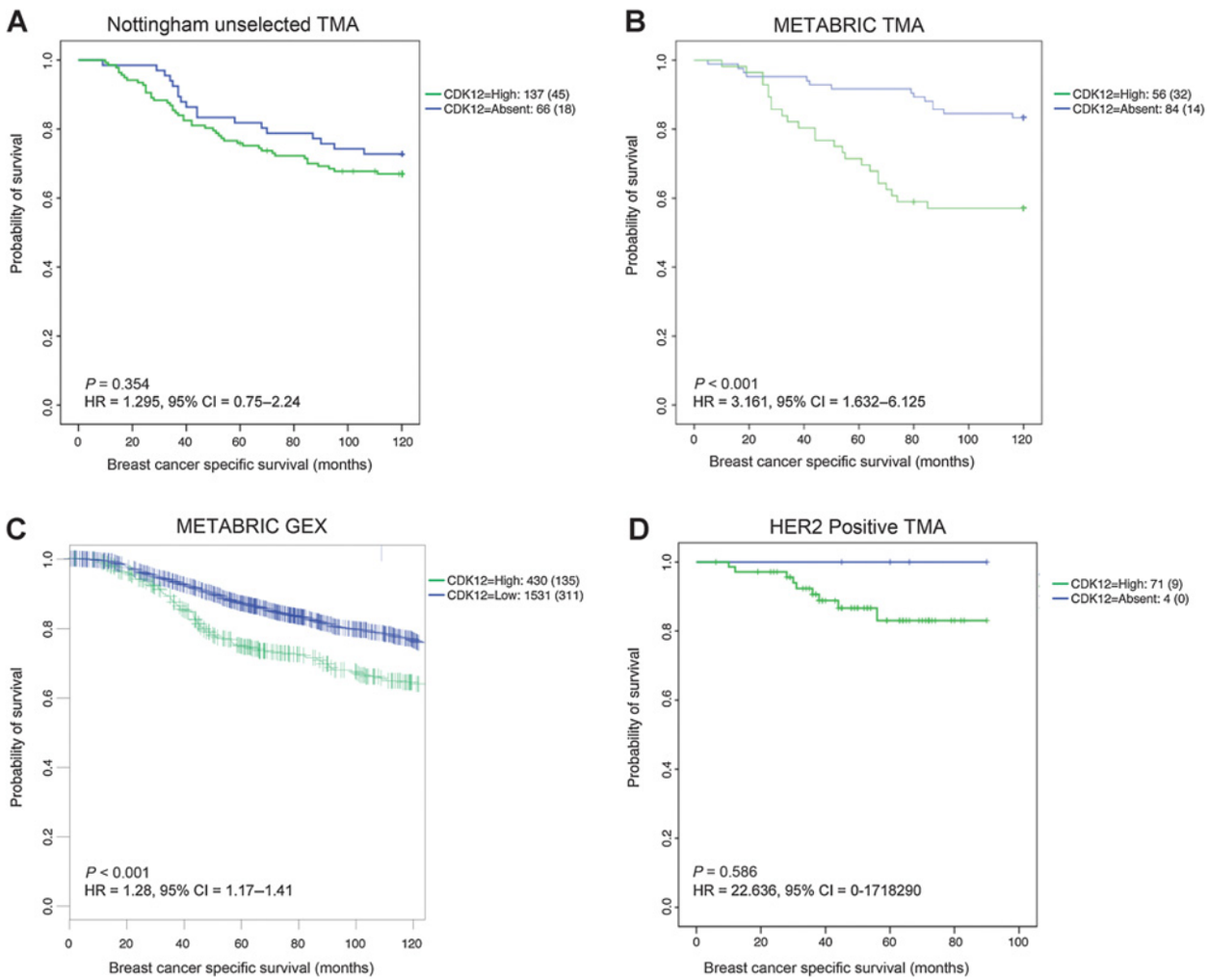

Figure 2.

CDK12-positive breast cancer has a poorer survival in univariate analysis. Kaplan–Meier curves showing breast cancer–specific survival for CDK12 high (7–8) versus absent (0) breast cancers asses assessed by IHC in the Nottingham unselected primary breast cancer series ($n = 203$; **A**); tumors from METABRIC ($n = 140$; **B**); Gene expression correlations of CDK12 low versus high from METABRIC ($n = 1{,}961$; **C**), and HER2-positive tumors treated with Herceptin ($n = 75$; **D**).

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with CDK12 fusions might also benefit from treatment with PARP1/2 inhibitors or platinum chemotherapy (13). To ascertain the frequency of CDK12 protein absence in HER2-amplified patients, and possible associations with outcome subsequent to anti-HER2 therapy, we assessed CDK12 protein expression in HER2-positive patients who had been treated with Herceptin (21). Overall, 4 of 119 (3.4%) tumors were CDK12 absent and 71 of 119 (59.7%) tumors were CDK12 high (Fig. 1A). Lack of CDK12 expression did not improve survival in this cohort following Herceptin treatment [P = 0.586; HR= 22.636; 95% CI = 0–1718290, log-rank (Mantel–Cox); Fig. 2D].

In summary (Supplementary Table S1), overall, absence of CDK12 protein expression was seen in 10.5% of unselected breast cancers; with a similar proportion of ER^+ tumors showing absent CDK12 (9.8%). Interestingly, a higher frequency of absent CDK12 was seen in TNBC within this unselected cohort (17%); however, no association with breast cancer–specific survival was seen [P = 0.577; HR= 2; 95% CI = 1.8–3.4, log-rank (Mantel–Cox)]. As expected, in all three cohorts analyzed, a lower proportion of HER2-positive tumors were CDK12 absent. Rather, most HER2-positive tumors were CDK12 high (protein and mRNA). Although high CDK12 expression was associated with a worse breast cancer–specific survival in these cohorts, this association was significant in the METABRIC dataset in multivariate analysis taking HER2 status into account, but not borne out in additional datasets.

Association of CDK12 expression and amplification

We subsequently investigated the associations of CDK12 mRNA expression with genomic status in primary breast cancer from METABRIC (23) where there is copy number and gene expression data on the same tumor specimen. Of 1,979 tumors with matched copy number and gene expression data available for CDK12, 208 (10.5%) harbored amplification encompassing the *CDK12* gene and a concurrent increase in its transcript expression, (P < 0.0001; Mann–Whitney U test; Fig. 3A). Of these, 99% (205/208) were also *HER2* amplified. There was a significant association with breast cancer–specific survival comparing *CDK12* amplified versus nonamplified tumors [P < 0.0001, log-rank (Mantel–Cox; HR = 0.45; 95% CI = 0.35–0.57]; however, similar to CDK12 protein expression, this was lost in multivariate analysis taking HER2 status into account (Supplementary Table S4). Assessment of CDK12 protein expression by IHC in the METABRIC TMA, stratifying tumors as absent (0) versus highly expressed (7–8), however, revealed a significant association between *CDK12* amplification and increased CDK12 protein expression with 12 of 16 (75%) amplified and 42 of 120 (35%) nonamplified tumors showing high protein expression, P = 0.0049, Fisher exact test, Fig. 3B).

Recent evidence has pointed to the role of *CDK12* as a potential oncogene, given that that its amplification is associated with increased protein expression and aggressive clinical characteristics (19) and that *CDK12*-amplified tumors show significantly increased CDK12 protein activation (39). To seek any evidence of oncogenic addiction to the downstream consequences of activated CDK12, we mined publicly available genetic perturbation screens in breast cancer cell line models using validated reagents (12, 13) to ascertain whether *CDK12*-amplified cells were addicted to CDK12 expression and downstream signaling for their survival. Analysis of both genome-wide pooled shRNA screen data (34) and siRNA kinome screen data (35) failed to identify any association between *CDK12* amplification and sensitivity to shRNA or siRNA designed to target *CDK12* (Fig. 3C and D).

Genetic mechanisms of absent CDK12 expression

Given our previous findings that disruption of *CDK12* can occur as a result of an amplification breakpoint in the *ERBB2* amplicon that converges on *CDK12*, disrupting its expression (13), we sought to confirm our findings in primary tumors from METABRIC (23). Of all cases that showed *CDK12* amplification, 14.4% (30/208) harbored a breakpoint in *CDK12* that was associated with a significant reduction in *CDK12* transcript levels (P < 0.0001; Mann–Whitney U test; Fig. 4A). In *HER2*-amplified patients, there was no significant difference in breast cancer–specific survival between patients with a breakpoint in *CDK12* and those without [P = 0.32; log-rank (Mantel–Cox) HR= 0.9897; 95% CI = 0.5107–1.918].

To ascertain the frequency of absent CDK12 protein in tumors with copy number alterations in *CDK12*, we intersected the IHC data from a subset of the METABRIC cohort performed above with the available copy number data for *CDK12*. Of all *CDK12*-amplified tumors, 14% (4/28) showed absent CDK12 protein expression and 33% (2/6) of tumors with a breakpoint in *CDK12* were CDK12 absent. This highlights that only a proportion of tumors with *CDK12* genomic breakpoints lead to loss of CDK12 protein expression.

In HGSOC, *CDK12*-inactivating mutations have been reported to inactivate gene expression and consequently abrogate HR DNA repair pathways (10, 11, 14). Examination of DNA sequencing data from The Cancer Genome Atlas (TCGA; ref. 32) identified *CDK12* mutations in 1.5% (20/1,373) of unselected breast cancer (Fig. 4B; Supplementary Table S5 and S6). These included 12 missense mutations, and 8 truncating mutations. Overall, 45% [9/20 (8 truncating and 1 missense] were predicted to disrupt protein function, and of these 25% (2/9) were seen in patients with triple-negative disease, whereas the remaining patients had ER^+ or $ER^-/HER2^+$ disease. In contrast with recent data in HGSOC (11), predicted deleterious mutations did not correlate with reduced transcript expression, or with downregulation of DDR genes, (Supplementary Table S7). CDK12 promoter methylation was a rare event seen in 1 of 696 (0.14%) of unselected breast cancer (Supplementary Table S6). We next looked for other genomic alterations that would lead to absent CDK12 protein expression by interrogation of METABRIC cases with copy number and miRNA expression (23, 40). This identified that heterozygous loss of CDK12 accounted for 7.1% (6/84) cases with absent CDK12 protein expression; however, this was not enriched in the CDK12-absent group compared with the CDK12-high group (P > 0.999, Fisher exact test). In addition, of the 14 of 162 miRNAs, that are known or predicted targets of CDK12 present in METABRIC (40), none showed correlation with CDK12 protein expression after multiple correction (Supplementary Table S8), suggesting there are additional mechanisms that lead to CDK12 protein loss.

Absent CDK12 protein expression is associated with reduced protein expression of genes involved in DNA repair

Although predicted deleterious mutations in *CDK12* did not correlate with reduced expression of DDR genes (see above), we

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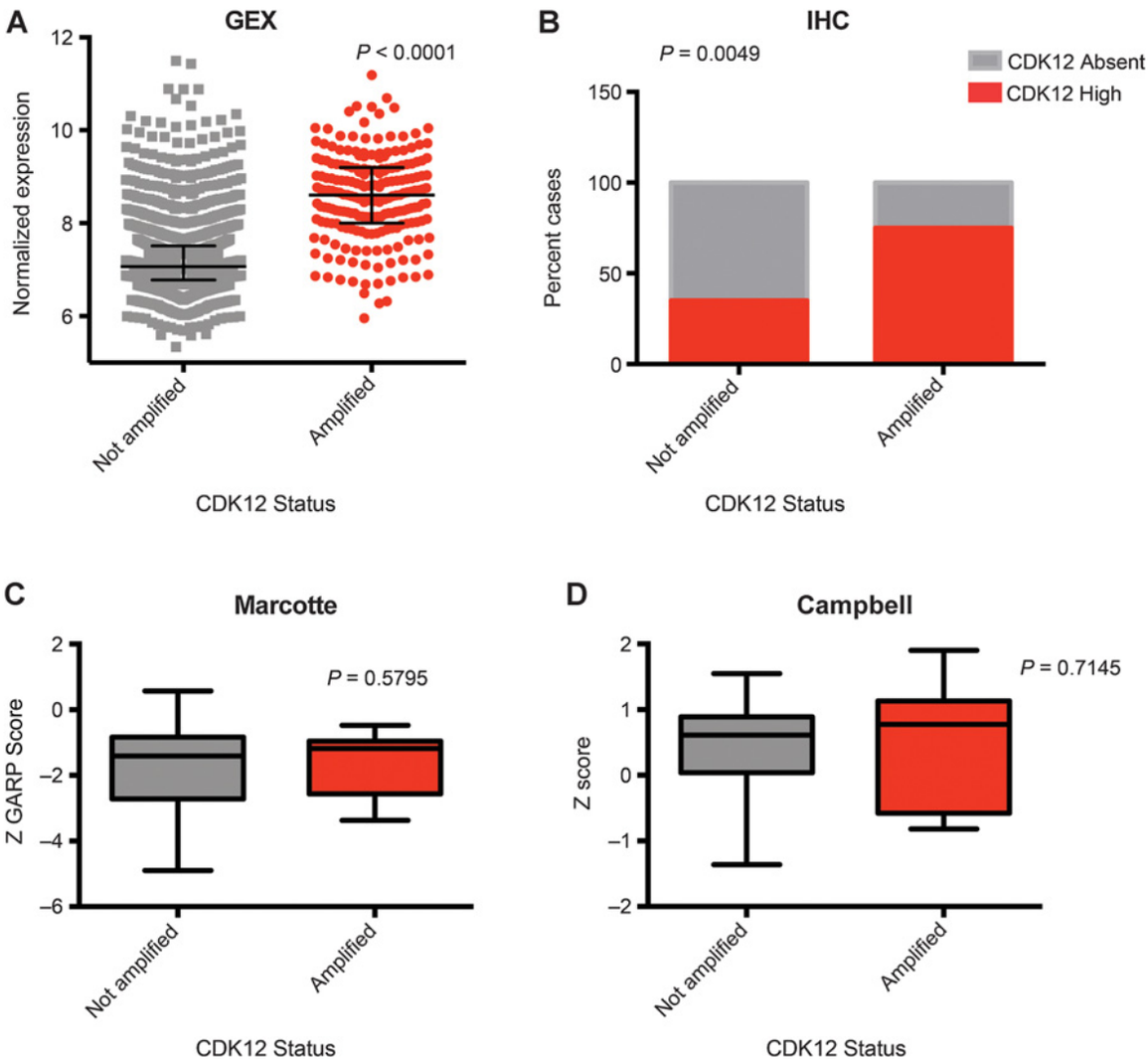

Figure 3.

CDK12-amplified cells are not dependent on CDK12 expression for their survival. **A,** Scatter dot plot depicting a significant association of *CDK12* transcript expression with gene amplification ($n = 208$) versus no amplification ($n = 1{,}769$; error bars represent median with the interquartile range). **B,** Bar chart depicting a significant increase of CDK12 protein expression as measured by IHC in *CDK12*-amplified tumors ($n = 16$) compared with nonamplified ($n = 103$). **C** and **D,** Relative cell viability after *CDK12* silencing in *CDK12* amplified (Campbell, $n = 8$; Marcotte $n = 14$) versus nonamplified cell lines (Campbell, $n = 19$; Marcotte $n = 40$), showing no significant difference in cell survival from Marcotte and colleagues (ref. 34; **C**) and Campbell and colleagues (ref. 35; **D**).

assessed whether loss of CDK12 protein expression correlated with a reduction in the transcript expression of DNA repair proteins or biomarkers of DNA damage in the unselected series of breast cancer cases (Table 1). Although no significant correlations were observed (after multiple testing correction) with DDR genes at the mRNA level (Supplementary Table S9), absent CDK12 protein was significantly correlated with reduced protein expression of ATR, APE1, nuclear and cytoplasmic SMC6, Ku70/Ku80, DNA-PK, and γH2AX nuclear positivity. Absent CDK12 protein expression also significantly correlated with both cleaved PARP1 ($P = 0.003$, χ^2) and noncleaved PARP1 ($P = 0.005$, χ^2) expression suggesting that PARP1 levels are higher in tumors with absent CDK12. Interestingly, absent CDK12 expression also correlated with a decreased expression of TP53 ($P = 0.001$; χ^2) and RB1 ($P = 0.003$, χ^2); however, there was no significant correlation between absent CDK12 protein and *TP53* mutations in the METABRIC cohort of tumors (17.5% CDK12 absent and 26.4% CDK12-high tumors harboring *TP53* mutations $P = 0.2779$, Fisher exact test).

As absent CDK12 protein expression was seen in 17% of TNBC within our analysis, we assessed this subset of tumors for correlations with the expression of DNA repair proteins as above. Even within this relative small subset, some significant correlations were still seen with DDR genes at the protein level: ATR ($P = 0.018$, χ^2); Ku70/Ku80 ($P = 0.01$, χ^2); and loss or decrease in nuclear and cytoplasmic SMC6 ($P = 0.045$, χ^2; Table 1). Of note,

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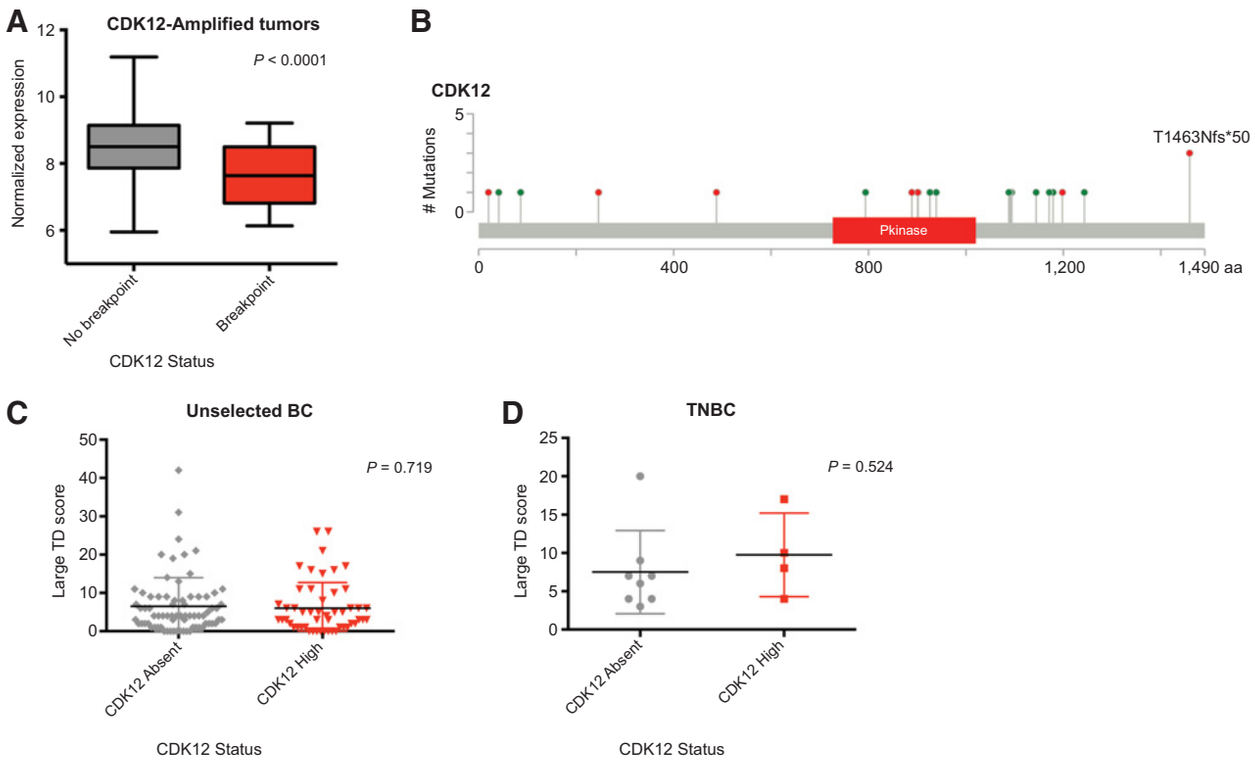

Figure 4.

CDK12 protein loss is associated with DNA repair defects in breast cancer. **A,** Box and whisker plot (min–max) showing a significant decrease in *CDK12* transcript levels in HER2-amplified tumors with breakpoints ($n = 30$) in *CDK12* compared with no breakpoints ($n = 178$) from METABRIC. **B,** Lollipop diagram depicting the distribution of *CDK12* mutations in breast cancer (red= frameshift and nonsense mutations, green= nonsynonymous coding mutations). **C,** Scatter dot plot diagrams showing significant associations between CDK12 absent ($n = 74$; IHC score 0) versus CDK12 high ($n = 51$; IHC score 7–8) in unselected METABRIC tumors with the large tandem duplication score, indicative of gross genomic defects. **D,** Scatter dot plot diagrams showing significant associations between CDK12 absent ($n = 8$; IHC score 0) versus CDK12 high ($n = 4$; IHC score 7–8) in TNBC from METABRIC, with the large tandem duplication score, indicative of gross genomic defects.

comparison of CDK12 absent (0) and intermediate (2–6) levels of expression together as one group versus high (7–8) failed to identify any significant associations with DNA repair genes both in unselected BC and TNBC (Supplementary Table S10). This suggests that functional loss of CDK12 is only observed in tumors with absent CDK12 protein expression in breast cancer and not low levels of CDK12 expression.

It has been shown that in HGSOC, CDK12 mutations are consistently associated with a particular genomic instability pattern characterized by hundreds of tandem duplications of up to 10 megabases (Mb) in size, dubbed the "CDK12 TD-plus phenotype" (14). Assessment of this pattern in both unselected and TNBC with absent CDK12 protein failed to identify any association with large numbers and sizes of tandem duplications (Fig. 4C and D). Together, these results suggest that absent CDK12 protein in breast cancer is associated with some defects in DNA repair–related genes; however, the resultant genomic scars are likely to be different to that seen in HGSOC (11).

Discussion

In this study, we were able to analyze, for the first time, the distribution of CDK12 protein in cohorts of primary breast cancer. We show that high CDK12 expression is significantly correlated with HER2 status and that absent CDK12 is associated with a triple-negative phenotype and disruption of proteins involved in DNA repair.

The significant correlation of CDK12 with HER2 expression in these data is not surprising, as CDK12 is known to map to the smallest region within the HER2 amplicon (17, 18). Therefore, although we observed significant correlations with CDK12 expression and patient survival, this significance was subsequently lost when HER2 positivity was taken into account. However, this association was substantiated in the METABRIC cohort at the protein level, suggesting investigation of additional cohorts is warranted. It has been postulated that CDK12 may act in an oncogenic manner, given observed associations with amplification and increased transcript and protein expression and subsequent phosphorylation (19, 39). This may perhaps occur through its reported roles in transcription through phosphorylation of RNA Pol II and regulation of pre-mRNA processing (5, 7). By analyzing published siRNA and shRNA cell viability screens, using validated reagents (12, 13), we show here that amplified cells are not addicted to CDK12 for their survival. We concede the assay length (average 4–7 days) in these experiments, may be too short to detect a loss of viability to depletion of a cyclin-dependent

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Table 1. Association of CDK12 expression with DNA repair proteins in unselected and TNBC. All *P* values are from χ^2 or Fisher exact test. *, $P < 0.05$; **, $P < 0.01$

	CDK12 Absent (0) *n* (%)	CDK12 High (7/8) *n* (%)	*P*
Unselected series			
BRCA1 ($n = 170$)			0.249
Negative	27 (51.9)	50 (42.4)	
Positive	25 (48.1)	68 (57.6)	
PARP1 cleaved ($n = 170$)			0.003**
Negative	18 (30)	13 (11.8)	
Positive	42 (70)	97 (88.2)	
PARP1 noncleaved ($n = 175$)			0.005**
Negative	36 (62.1)	46 (39.3)	
Positive	22 (37.9)	71 (60.7)	
ATR ($n = 173$)			<0.001**
Negative	46 (70.8)	45 (41.7)	
Positive	19 (29.2)	63 (58.3)	
APE1 ($n = 89$)			0.02*
Negative	10 (38.5)	10 (15.9)	
Positive	16 (61.5)	53 (84.1)	
Ku70/Ku80 ($n = 153$)			0.001**
Negative	18 (34.0)	11 (11.0)	
Positive	35 (66.0)	89 (89.0)	
DNA-PK ($n = 156$)			0.002**
Negative	17 (32.1)	12 (11.7)	
Positive	36 (67.9)	91 (88.3)	
SMC6 cytoplasmic ($n = 166$)			0.002**
Negative	30 (54.5)	33 (29.7)	
Positive	25 (45.5)	78 (70.3)	
SMC6 nuclear ($n = 166$)			<0.001**
Negative	33 (60.0)	34 (30.6)	
Positive	22 (40.0)	77 (69.4)	
γH2AX ($n = 150$)			0.002**
Negative	24 (47.1)	22 (22.2)	
Positive	27 (52.9)	77 (77.8)	
TP53 ($n = 209$)			0.001**
Negative	56 (80.0)	80 (57.6)	
Positive	14 (20.0)	59 (42.4)	
CHEK1 cytoplasmic ($n = 103$)			0.321
Negative	29 (50.9)	44 (42.7)	
Positive	28 (49.1)	59 (57.3)	
CHEK1 nuclear ($n = 161$)			0.927
Negative	48 (84.2)	87 (83.7)	
Positive	9 (15.8)	17 (16.3)	
CHEK2 ($n = 108$)			0.345
Negative	31 (50.8)	63 (58.3)	
Positive	30 (49.2)	45 (41.7)	
Triple-negative breast cancer			
BRCA1 ($n = 28$)			0.430
Negative	10 (71.4)	8 (57.1)	
Positive	4 (28.6)	6 (42.9)	
PARP1 cleaved ($n = 28$)			0.378
Negative	8 (50.0)	4 (33.3)	
Positive	8 (50.0)	8 (66.7)	
PARP1 noncleaved ($n = 29$)			0.103
Negative	11 (68.8)	5 (38.5)	
Positive	5 (31.2)	8 (61.5)	
ATR ($n = 33$)			0.018*
Negative	18 (90.0)	7 (53.8)	
Positive	2 (10.0)	6 (46.2)	
APE1 ($n = 17$)			0.453
Negative	2 (25.0)	1 (11.1)	
Positive	6 (75.0)	8 (88.9)	
Ku70/Ku80 ($n = 27$)			0.010*
Negative	8 (47.1)	0 (0.0)	
Positive	9 (52.9)	10 (100.0)	
DNA-PK ($n = 31$)			0.283
Negative	6 (37.5)	3 (25.0)	
Positive	10 (62.5)	12 (75.0)	
SMC6 cytoplasmic ($n = 31$)			0.018*
Negative	9 (60.0)	3 (23.1)	
Positive	6 (40.0)	13 (76.9)	
SMC6 nuclear ($n = 31$)			0.045*
Negative	11 (73.3)	6 (37.5)	
Positive	4 (26.7)	10 (62.5)	
γH2AX ($n = 29$)			0.089
Negative	9 (60.0)	4 (28.6)	
Positive	6 (40.0)	10 (71.4)	
TP53 ($n = 36$)			0.187
Negative	12 (63.2)	7 (41.2)	
Positive	7 (36.8)	10 (58.8)	
CHEK1 cytoplasmic ($n = 27$)			0.384
Negative	8 (47.1)	3 (30.0)	
Positive	9 (52.9)	7 (70.0)	
CHEK1 nuclear ($n = 28$)			0.068
Negative	17 (100.0)	9 (81.8)	
Positive	0 (0.0)	2 (18.2)	
CHEK2 ($n = 32$)			0.961
Negative	13 (68.4)	9 (69.2)	
Positive	6 (31.6)	4 (30.8)	

(Continued on the following column)

Table 1. Association of CDK12 expression with DNA repair proteins in unselected and TNBC. All *P* values are from χ^2 or Fisher exact test. *, $P < 0.05$; **, $P < 0.01$ (Cont'd)

kinase, and additional time may be required to reduce DNA repair protein expression and accumulate damage required to affect viability. Interestingly, a recent study has suggested that CDK12 does act in an oncogenic manner in HER2-amplified cells by promoting cell invasion via alternative splicing and subsequent downregulation of the long isoform of DNAJB6 (41), suggestive of a mechanism by which CDK12 acts in an oncogenic manner to promote cell invasiveness. Of note, elevated levels of CDK12 in HER2-positive tumors have been a proposed reason why tumors arising in *BRCA1* carriers are usually ERBB2-negative, given elevated expression would oppose the defects in HR mediated by *BRCA1* loss (34).

We have shown previously that a proportion of HER2-positive tumors harbor inactivating (out-of-frame) fusion genes in *CDK12* that are due to a copy number breakpoint in the HER2 amplicon converging on *CDK12*, resulting in a significant decrease in both transcript and protein levels (13). Furthermore, cell lines with breakpoints in *CDK12* that result in loss of protein expression are sensitive to PARP1/2 inhibitor therapy, due to impaired HR-mediated DNA repair (13). By assessing the distribution of CDK12 protein expression in CDK12-amplified breast cancers in the METABRIC cohort, we identified 14% that were CDK12 absent. Moreover, of the tumors with a breakpoint in CDK12, 33% had absent CDK12 protein expression. While the numbers we were able to assess are small, this nevertheless suggests a proportion of HER2-positive patients show absent CDK12 protein as a result of both copy number breakpoints and additional mechanisms. Overall, our data suggest there may be a small proportion of HER2-positive patients that may benefit from treatment with DNA damage response–targeted therapies such as PARP1/2 inhibitor therapy.

Recent data in HGSOC has shown that *CDK12* point mutations inactivate gene expression subsequently abrogating HR DNA repair pathways (10, 11). Although in our study *CDK12* mutations did not themselves correlate with decreased expression of

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DNA repair genes (perhaps due to residual CDK12 activity, given some of these are not associated with loss of heterozygosity), we show that breast cancer with absent CDK12 protein show downregulation of a number of genes involved in functional HR DNA repair at the protein level, suggesting that absent CDK12 protein in breast cancer may also be associated with HR defects. Although we were unable to assess all proteins known to be dysregulated in CDK12-mutant HGSOC such as RAD51, FANCI, and FANCD2, it is reasonable to postulate that CDK12 may have other targets, including DNA repair proteins themselves, phosphorylation of which is required for protein stability. Lack of any observed associations at the mRNA level, may be due to small numbers in the study, however, our observed associations warrant further investigation in larger cohorts. Recent evidence in HGSOC points to a unique signature of genomic instability in *CDK12*-mutated tumors that is indicative of gross defects in DNA repair characterized by tens to hundreds of large tandem duplications scattered throughout the genome, although this was not observed in *CDK12*-mutated breast cancers (14). Consistent with this, we observed no correlation with large tandem duplications of neither all breast cancer nor TNBC specifically. It is intriguing, however, that we observed absent CDK12 protein in 17% of TNBC from the unselected primary breast cancer analyzed in this study. Of course, this could be a consequence of the small number of cases included; however, CDK12 inactivation may play a role in a subset of TNBC's possibly through mechanisms distinct from those observed in HGSOC.

In conclusion, we have shown that a subset of HER2-positive patients show absent CDK12 protein expression and have shown an enrichment of absent CDK12 protein expression in TNBC. Moreover, we have provided evidence that absent CDK12 expression is associated with defects in DNA repair proteins. These results suggest that CDK12 IHC could potentially be useful, once validated in sufficiently powered studies, for stratification of patients for treatment with DDR-targeted therapies.

Disclosure of Potential Conflicts of Interest

C.J. Lord is a named inventor on patents describing the use of PARP inhibitors and stand to gain from their use as part of the ICR "Rewards to Inventors" scheme. No potential conflicts of interest were disclosed by the other authors.

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Molecular Cancer Therapeutics

Evaluation of CDK12 Protein Expression as a Potential Novel Biomarker for DNA Damage Response–Targeted Therapies in Breast Cancer

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