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Knowlson, Catherine orcid.org/0000-0001-5783-2690, Haddock, Paula, Bingham, Victoria et al. (3 more authors) (2020) Pin1 plays a key role in the response to treatment and clinical outcome in triple negative breast cancer. Therapeutic advances in medical oncology. ISSN 1758-8340

https://doi.org/10.1177/1758835920906047

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Original Research

Pin1 plays a key role in the response to treatment and clinical outcome in triple negative breast cancer

Ther Adv Med Oncol

2020, Vol. 12: 1–12

DOI: 10.1177/1758835920906047

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Abstract

Background: Triple negative breast cancer (TNBC) is the subset of breast cancer associated with the poorest outcome, and currently lacks targeted treatments. Standard of care (SoC) chemotherapy often consists of DNA damaging chemotherapies ± taxanes, with a range of responses observed. However, we currently lack biomarkers to predict this response and lack alternate treatment options.

Methods: Pin1 expression was modulated *in vitro* and proliferation and treatment response was studied. Pin1 expression was analysed in patient samples and correlated with clinical outcome.

Results: In this study, we have shown that the prolyl isomerase, Pin1, which is highly expressed in TNBC, plays a key role in pathogenesis of the disease. Knockdown of Pin1 in TNBC resulted in cell death while the opposite is seen in normal cells. We revealed for the first time that loss of Pin1 leads to increased sensitivity to Taxol but only in the absence of functional BRCA1. Conversely, loss of Pin1 results in decreased sensitivity to DNA-damaging agents independent of BRCA1 status. Analysis of Pin1 gene or IHC-based expression in over 200 TNBC patient samples revealed a novel role for Pin1 as a TNBC-specific biomarker, with high expression associated with improved outcome in the context of SoC chemotherapy. Preliminary data indicated this may be extended to other treatment options (e.g. Cisplatin/Parp Inhibitors) that are gaining traction for the treatment of TNBC.

Conclusions: This study highlights the important role played by Pin1 in TNBC and highlights the context-dependent functions in modulating cell growth and response to treatment.

Keywords: biomarker, BRCA1, chemotherapy, Pin1, triple negative breast cancer

Received: 16 September 2019; revised manuscript accepted: 22 January 2020.

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Introduction

Triple negative breast cancer (TNBC) is defined based on the lack of the expression of the estrogen (ERα) and progesterone (PR) receptors, as well as the absence of HER2 amplification. As this is a diagnosis of exclusion, TNBC is a highly heterogeneous subgroup of breast cancer with poor outcome. While numerous studies have aimed to further stratify TNBC in order to tailor treatment (reviewed in Bianchini and colleagues)[1], to date these have not resulted in a change in standard of care (SoC); most patients receive DNA-damaging chemotherapy ± taxanes in the adjuvant and, more recently, the neo-adjuvant setting.[2,3] While some patients respond very well to this treatment regimen, there is still a significant proportion of patients who receive little clinical benefit, relapse and die from their disease in a short period of time.[4] Therefore, there is a significant unmet clinical need to identify biomarkers that allow TNBC to be stratified based on knowledge of the underlying biology and for treatment options to be tailored accordingly.

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We and others have shown that the prolyl isomerase, Pin1, is transcriptionally repressed by BRCA1.[5,6] Furthermore, we have shown that high Pin1 levels, as observed in the absence of functional BRCA1, results in increased activity of the Src family kinase, Lyn.[5] This leads to increased migration and invasion, key features of aggressive breast cancer. Given the strong link between BRCA1 dysfunction (BRCAness) and TNBC,[7] as well as association of Pin1 with poor prognostic factors such as high grade,[8] we thought it pertinent to investigate the role of Pin1 in TNBC and the potential therapeutic implications.

Materials and methods

Cell lines

All cell lines have been described before,[9] with the exception of the HCC3153 cells which were obtained from Adi Gazdar (UT Southwestern, Dallas, TX, USA). Cell lines were characterized by isoenzyme/cytochrome *c* oxidase I (COI) assay and short tandem repeat (STR) analysis by ATCC.

Growth assays and dose response curves

Cells were pretreated with short interfering RNA (siRNA) for 24 h before reseeding at an optimized cell density. For growth assays, cells were stained with crystal violet and quantified at an absorbance of 570 nm following reabsorption with sodium citrate. For dose response curves, cells were treated with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT; Sigma, St. Louis, MO, USA) for 3–4 h, 72 h post drug treatment. Crystals were reabsorbed with DMSO and quantified at an absorbance of 570 nm. All chemotherapies were obtained from the Belfast City Hospital Pharmacy. UMI-77 was purchased from Cayman Chemicals (Ann Arbor, MI, USA) and Olaparib was purchased from Axon MedChem (Groningen, The Netherlands).

Short interfering RNA

Transfections were done using RNAiMax reagent (Invitrogen, UK), as outlined in the manufacturer's instructions. siRNA oligonucleotides were obtained from Eurofins and used at a final concentration of 10 nM. Scr: AAGCAGCACGACT TCTTCAAG and Pin1: CTGGCCTCACAGTT CAGCG and GCTCAGGCCGAGTGTACTA

Western blot analysis

Protein lysates were extracted in EDTA Lysis Buffer (ELB) (0.25 M NaCl, 0.1% IEPGAL, 0.25 M Hepes, 5 mM EDTA, 0.5 mM DTT), separated on a SDS PAGE gel, transferred to a PVDF membrane followed by immunoblotting. Antibodies were purchased from SantaCruz (Pin1- sc15340, GAPDH - sc32233, Mcl-1 - sc12765, CtIP - sc271339, BRCA1 -sc6954 and Cleaved Caspase 3 - sc56055) and Cell Signalling [Chk2–2662 and 2197 (T68)] (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

RNA extraction, reverse transcription and real-time quantitative PCR

RNA was extracted using RNA STAT60 Total RNA extraction reagent (Tel-Test Inc, Friendswood, TX, USA), reverse transcribed using the Transcriptor First Strand cDNA Synthesis kit (Roche, Welwyn Garden City, UK) and real-time quantitative PCR (RqPCR) analysis performed on the LC96 (Roche) using Sybr Green (Roche) according to the manufacturer's instructions. Primers used were; Pin1: F GAAGATCACCCGG ACCAAG, R AAGTCCTCCTCTCCCGACTT; HPRT1: F TGACCTTGATTTATTTTGCA TACC, R CGAGCAAGACGTTCAGTCCT; ACTB: F TCCTCCCTGGAGAAGAGCTA, R CGTGGATGCCACAGGACT

Pin1 immunohistochemistry

Tissue microarrays (TMAs) were obtained from the Northern Ireland Biobank as previously described,[10] and the Breast Cancer Now Tissue Bank with ethical approval (NIB12-0043 and TR-00055, respectively). Both biobanks have ethical approval to use deidentified tissue samples from the NHS tissue pathology archives with matched deidentified data. In accordance with the Human Tissue Act, consent is not required for use of archived, deidentified tissue in research studies with ethical approval.

The Breast Cancer Now Tissue Bank TMA was constructed from formalin-fixed paraffin-embedded (FFPE) primary block by the biobank, with each tumour sample represented by three independent 1 mm diameter cores. These were obtained from 115 TNBC patients undergoing surgery and SoC treatment between 1988 and 2014. All patients were Grade 3 with a median age of 45 (Range 28–96) and median follow-up of

8 years. Both TMA cohorts were powered to detect a minimum Hazard ratio of 2 with 80% power (alpha = 0.05) based on an event rate of ~30%, which is normally observed in TNBC.

Immunohistochemistry (IHC) was performed in a hybrid laboratory (Northern Ireland Molecular Pathology Laboratory) that has UK Clinical Pathology Accreditation, and the infrastructure to process both clinical patient samples and research materials. Sections were cut from the TMA blocks for Hematoxylin and eosin (H&E) staining and IHC. The initial section was used for H&E staining to assess TMA quality and appropriate tumour content for subsequent IHC localization and analysis. Sections for IHC were cut at 4 µm on a rotary microtome, dried at 37°C overnight, and then used for IHC, performed on an automated immunostainer (Leica Bond-Max, Milton Keynes, UK). Repeat ER, PR and HER2 IHC were performed to confirm the triple negative status all samples in the TMA as previously described.[11] The Pin1 antibody was validated in house using positive and negative whole-face breast cancer sections identified through gene expression before the TMAs were stained. Antigen-binding sites were detected with a polymer-based detection system (Bond, Newcastle Upon Tyne, UK, Cat. No. DS 9800). All sections were visualized with diaminobenzidine, counterstained with haematoxylin, and mounted in DPX. Biomarker conditions were as follows. Pin1 (sc-46660) was used at a 1:200 with epitope retrieval solution 1 pretreatment for 20 mins.

Only cores with identifiable tumour as confirmed by pathology assessment of H&E slides were used in IHC analysis. All IHC was scored independently by at least two experienced immunohistochemists blinded to patient clinicopathological and outcome data.

Survival analysis and statistics

All survival analysis (Relapse and Overall) and statistical analysis was carried out using GraphPad Prism (v8.2).

Results

We first investigated Pin1 expression using a publicly available RNASeq dataset of 77 breast cancer cell lines.[12] Pin1 expression varied significantly ($p = 0.0396$) across breast cancer subtypes defined by the three gene (ER/PR/HER2) classifier, with the highest expression observed in TNBC (Figure 1a(i)). Examination of TNBC in more detail showed a range of expression across the cell lines [Figure 1a(ii) and Supplementary Table S1], with no significant correlation observed between Pin1 expression and the molecular subtypes of breast cancer defined by Lehmann and colleagues [Figure 1a(iii)].[13] Consistent with the *in silico* findings, when Pin1 protein and mRNA expression was studied in a panel of TNBC cell lines, Pin1 was detected in all cell lines tested with highest expression in the BRCA1-low MDA-468 cells (Figure 1b and c).[14]

We next wanted to understand the functional significance of the high Pin1 expression observed in TNBC. In order to do this, we first knocked down Pin1 by siRNA in TNBC cell lines and measured cell growth. Pin1 siRNA resulted in a significant decrease in cell growth in all cell lines studied (Figure 2a). This was associated with an increase in cleaved caspase 3 (Figure 2b) indicating this is mediated by apoptotic cell death. Interestingly, knockdown of Pin1 in the normal breast cell lines, HME1 and 184A1 lead to a significant increase in cell growth (Figure 2c and Supplementary Figure S1).

Next, we wanted to investigate the role of Pin1 in response to chemotherapy, as Pin1 has been linked to drug resistance and chemotherapy is SoC for TNBC.[15] We first examined the effect of Pin1 knockdown on the cellular response to the antimicrotubule agent Taxol (Paclitaxel) using dose response curves. While no difference was observed in BRCA1-proficient cell lines (Figure 3a, Supplementary Figure S2a and Supplementary Table S2a), loss of Pin1 expression was associated with a significant increase (~10 fold) in sensitivity to Taxol in the BRCA1 mutant HCC-1937 and Sum149 cell lines (Figure 3b). In order to investigate this further, the isogenic MDA-468 cell line, where wildtype BRCA1 is overexpressed in the BRCA1-low MDA-468 cell line, was utilized.[14] As we have shown previously,[5] BRCA1 expression represses the expression of Pin1 [Supplementary Figure S2b(i)]. Consistent with the known role of BRCA1 in the cellular response to antimicrotubule agents,[16,17] overexpression of BRCA1 results in a significant increase in sensitivity to Taxol (IC_{50} 71 nM *versus* 7 nM). Knockdown of Pin1 with two independent siRNA sequences also resulted in a significant increase in cell death and sensitivity to Taxol but only in the MDA468 EV BRCA1 low cell line [Figure 3c,

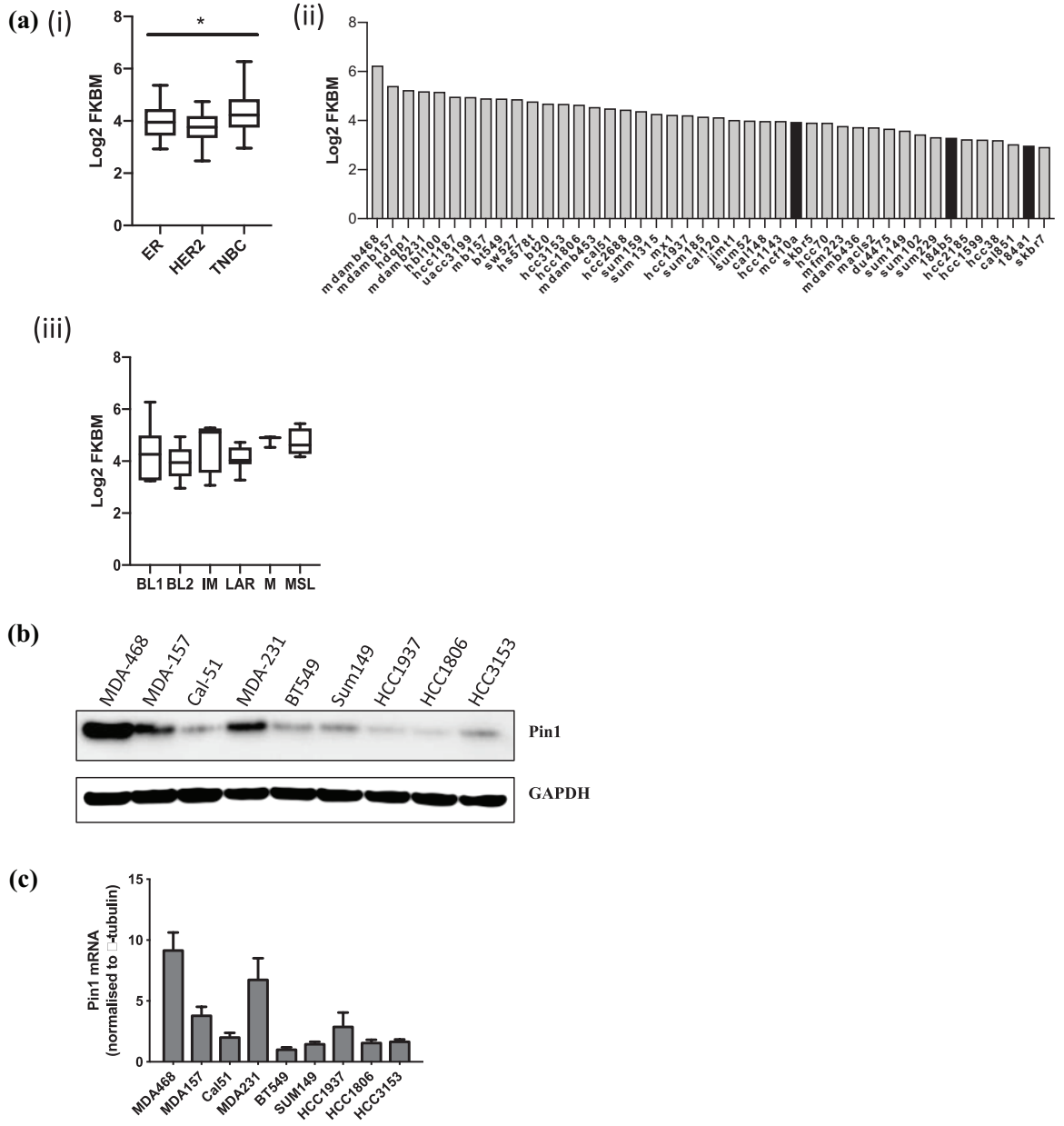

Figure 1. (a) RNASeq-based Pin1 expression in breast cell lines categorized by 3 gene classifier (i), TNBC cell line (with normal cell lines indicated in black) (ii) and Lehman TNBC subtype (iii) from the publicly available GSE73526. Variance was tested by one-way ANOVA ($p = 0.0396$). (b) Western blot of a panel of TNBC cell lines probed with Pin1 and GAPDH as a housekeeper. (c) Real-time PCR analysis of Pin1 mRNA expression in the same panel of TNBC cell lines as (b). The housekeeper gene β-tubulin was used as a loading control.
GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PCR, polymerase chain reaction; TNBC, triple negative breast cancer.

Supplementary Figure S2b(ii), 2c and Supplementary Table S2b]. We hypothesized that differential effect may be mediated by the antiapoptotic Bcl-2 family member, Mcl-1, which has been shown to play a key role in the cellular response to Taxol.[18] Furthermore, Pin1 has been shown to modulate this response through stabilization of the Mcl-1 protein. Consistent with this, knockdown of Pin1 resulted in loss of Mcl-1 expression in untreated cells and Taxol treatment resulted in loss of Mcl-1 protein expression in the MDA468 BR cells. Conversely, in the absence of functional BRCA1, where high Pin1 levels are present, Mcl-1 levels remain high and are associated with

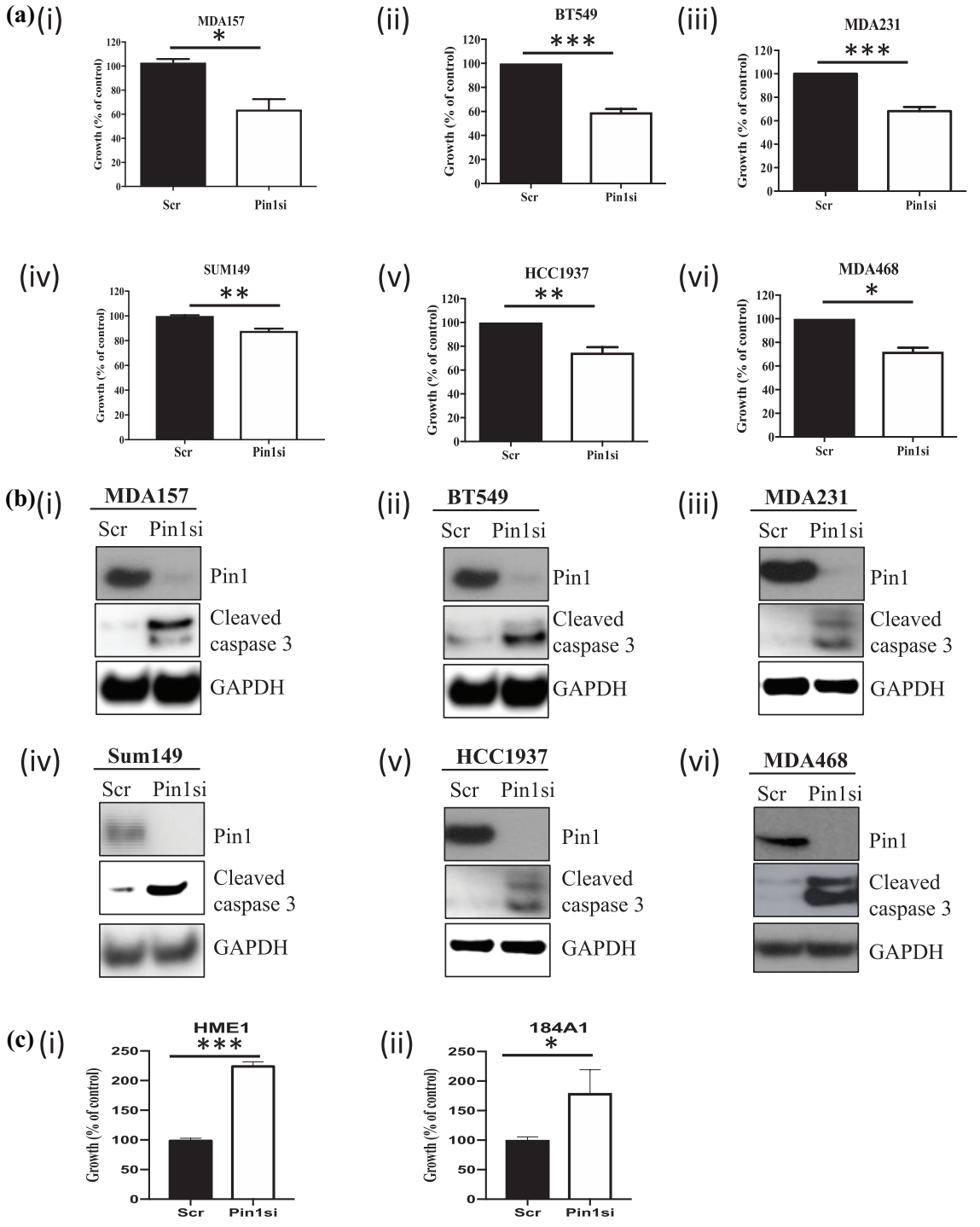

Figure 2. (a) Growth assays of (i) MDA157, (ii) BT549, (iii) MDA231, (iv) SUM149, (v) HCC1937 and (vi) MDA468 cells pretreated with either Pin1 or Scr siRNA as a control. After 5–7 days, cells were stained with crystal violet, reabsorbed and quantified at A570 nm. Cell growth was normalized to Scr control ($p = 0.0126$, 0.0001, 0.0009, 0.0032, 0.0052 and 0.0138 respectively). (b) Western blot of the same cells as (a) treated with either Pin1 or Scr siRNA for 72 h. Blots were then probed with Pin1, Caspase-3 and GAPDH as a loading control. (c) Growth assays of (i) HME1 and (ii) 184A1 cells pretreated with either Pin1 or Scr siRNA as a control. After 5–7 days, cells were stained with crystal violet, reabsorbed and quantified at A570 nm. Cell growth was normalized to Scr control ($p \leqslant 0.0001$ and 0.0271 respectively).

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Scr, Scrambled; siRNA, short interfering RNA

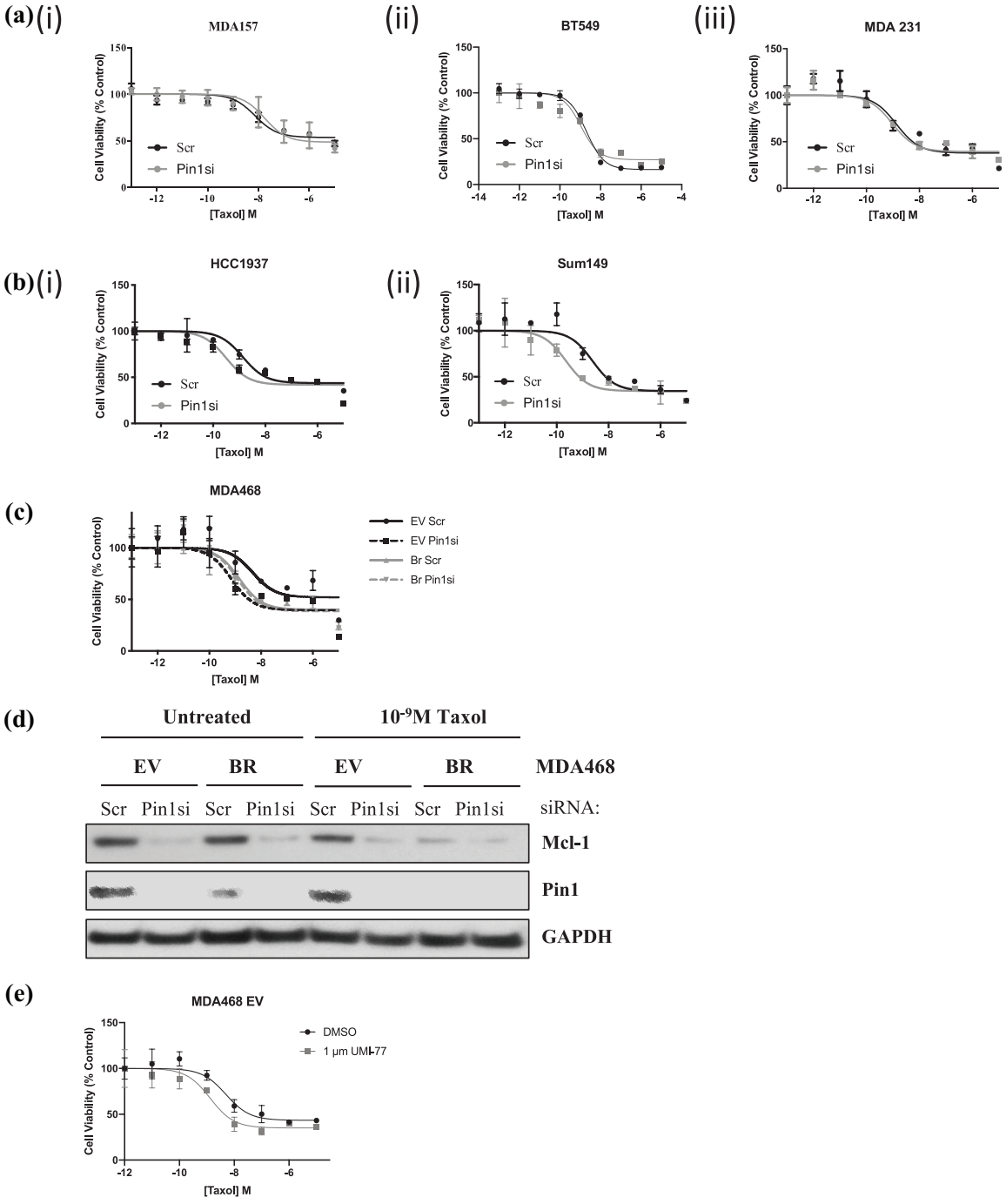

Figure 3. Dose response curve of (a) (i) MDA157, (ii) BT549, (iii) MDA231, (b) (i) SUM149 and (ii) HCC1937 cells pretreated with either Pin1 or Scr siRNA for 24 h before treatment with a range of concentrations of Taxol for 72 h. Cell viability was then assessed by MTT with cell survival normalized to vehicle control (100%). (c) Dose response curve of the BRCA1-low MDA468 cells stably transfected with BR or EV control pretreated with either Pin1 or Scr siRNA for 24 h before treatment with a range of concentrations of Taxol for 72 h. Cell viability was then assessed by MTT with cell survival normalized to vehicle control (100%). (d) Western blot of the BRCA1-low MDA468 cells stably transfected with BR or EV control pretreated with either Pin1 or Scr siRNA for 24 h before treatment with vehicle control or 1nMTaxol for 24 h. Blots were then probed with Mcl-1, Pin1 and GAPDH as a loading control. (e) Dose response curve of MDA468 EV cells pretreated with 1 μM of the Mcl-1 inhibitor, UMI-77, before treatment with a range of concentrations of Taxol for 72 h. Cell viability was then assessed by MTT with cell survival normalized to vehicle control (100%).
BR, BRCA1; EV, empty vector; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; Scr, Scrambled.

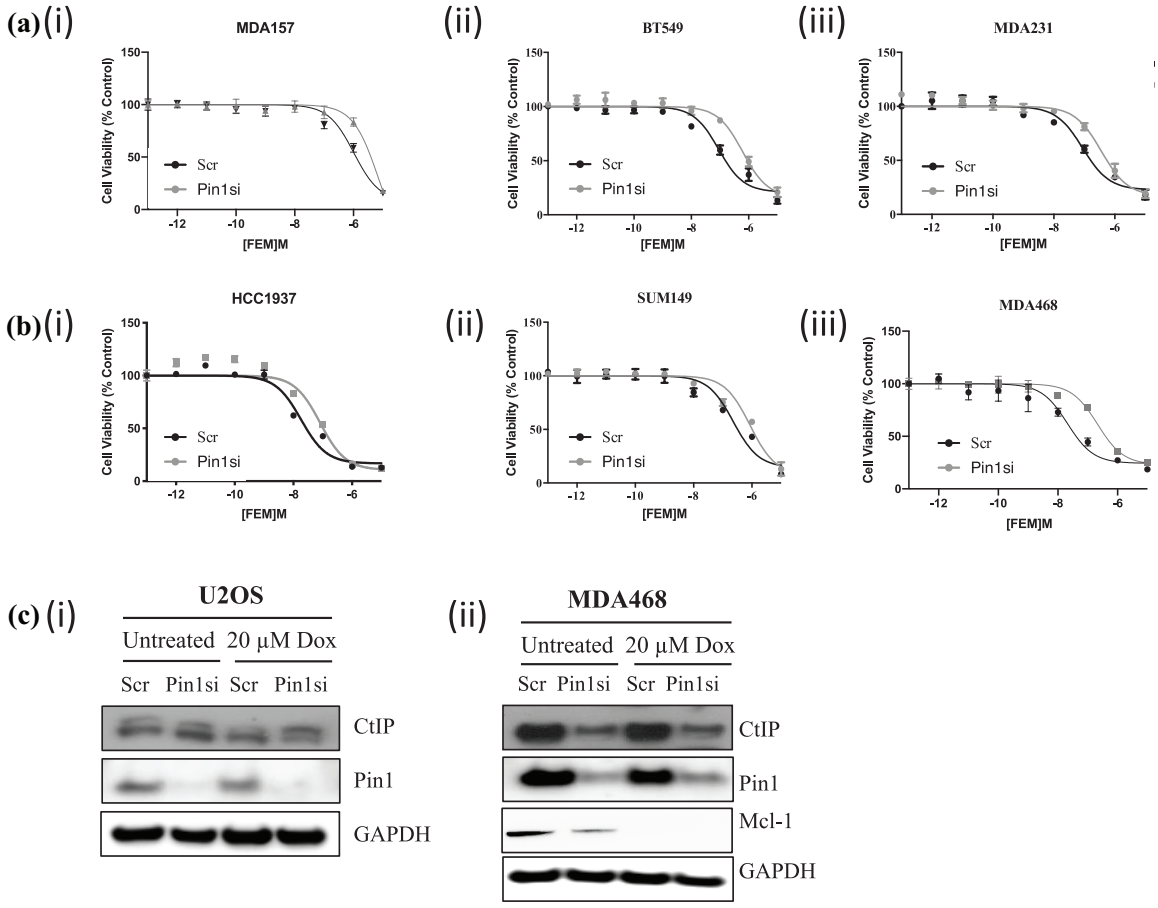

Figure 4. Dose response curves of (a) (i) MDA157, (ii) BT549, (iii) MDA231, (b) (i) SUM149 (ii) HCC1937 and (iii) MDA468 cells pretreated with either Pin1 or Scr siRNA for 24 h before treatment with a range of concentrations of a cocktail of 5-FU, Epirubicin and Mitomycin C (FEM)for 72 h. Cell viability was then assessed by MTT with cell survival normalized to vehicle control (100%). (c) Western blot of (i) U2OS and (ii) MDA468 cells pretreated with either Pin1 or Scr siRNA for 24 h before treatment with vehicle control or 20 μM Dox for 6h. Blots were then probed with CtIP, Pin1, Mcl-1 and GAPDH as a loading control. The upper CtIP band represents hyperphosphorylated CtIP.
DOX, doxorubicin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; Scr, Scrambled.

resistance to treatment (Figure 3d). In order to test this hypothesis further, we utilized the Mcl-1 inhibitor, UMI-77,[19] and showed a ninefold increase in sensitivity to Taxol in the MDA468 EV cells line compared with that observed with Pin1 siRNA (Figure 3e and Supplementary Table S2c).

We next wanted to investigate the role of Pin1 in the response to DNA-damaging chemotherapy utilizing an FEC-like cocktail, FEM, to mimic SoC (5-FU, Epirubicin and Mitomcyin C replacing Cyclophosphamide, which required metabolic activation *in vivo*). In contrast to what was observed in the context of Taxol, Pin1 siRNA resulted in a decrease in sensitivity to FEM as shown by dose response curve and cleaved caspase 3 western blot in both BRCA1 proficient [Figure 4a and Supplementary Figure S3a(i)] and deficient cell lines [Figure 4b, Supplementary Figure S3a(ii) and Supplementary Table S3a]. Similar results were also observed with a second siRNA sequence (Supplementary Figure S3b and c). Pin1 has previously been linked to double-strand break repair, with overexpression of Pin1 suppressing HR though destabilization of CtIP.[20] While we were able to recapitulate the findings of Steger and colleagues in the U20S cell line [Figure 4c(i)],[20] increased phosphorylation of CtIP (a key event in DSB repair) following Pin1 siRNA and treatment with the DNA-damaging agent, Doxorubicin, was not observed in the MDA-468 cell line despite the increase in resistance (Supplementary Figure S3c and Supplementary Table S3b). In fact, an overall decrease in CtIP levels

was observed [Figure 4c(ii)]. As expected, Mcl-1 was downregulated by treatment with doxorubicin as anthracyclines are known to be global repressors of transcription that preferentially impact Mcl-1, given the short half-life of the mRNA.[21] This was not modulated by Pin1 expression [Figure 2c(ii)], indicating that there may be multiple mechanisms underpinning the modulation of DNA repair by Pin1.

Given the role of Pin1 in multiple cancer-associated phenotypes, we next wanted to investigate whether Pin1 expression could be used as a biomarker to predict outcome in patients with TNBC. Previous published data showed that Pin1 was associated with high grade and poor prognosis breast cancer.[8,22,23] This was recapitulated in the analysis of a largely untreated TNBC cohort,[24] showing that high Pin1 mRNA was significantly associated with decreased relapse-free survival [hazard ratio (HR) 1.7; 95% confidence interval (CI) 1.26–2.3; $p=0.0005$] [Figure 5A(i) and Supplementary Table S4A]. However, given the role of Pin1 in DNA repair, we hypothesized that Pin1 may play an alternative role as a predictive biomarker to DNA-damaging chemotherapy. Indeed, analysis of an FEC-treated TNBC cohort showed that high Pin1 mRNA expression was associated with improved relapse free survival,[9] although this did not quite reach significance ($p=0.0747$) [Figure 5A(ii) and Supplementary Table S4A]. To explore this further, we carried out Pin1 IHC on two independent TNBC cohorts, both treated with SoC chemotherapy. A range of expression of Pin1 was observed scored as absent (0), low (1), intermediate (2), high (3) and extremely high (4) expression (Figure 5b). When present, Pin1 was expressed in both the cytoplasm and the nucleus, consistent with its known patterns of localization.[25] Pin1 expression was then correlated with relapse-free and overall survival. Based on preliminary investigations, patients were stratified based on low (Score 0 and 1) and high (Score 2–4) Pin1 expression. Consistent with the gene expression data, high Pin1 was significantly associated with improved relapse-free and overall survival in both the Northern Ireland and Breast Cancer Now Biobank cohorts of patients treated with SoC DNA Damaging chemotherapy (Figure 5c and d and Supplementary Table S4B). This appeared to be TNBC specific, as analysis of a larger cohort of FEC-treated breast cancer cases representing all molecular subgroups of breast cancer showed no differences on survival based on Pin1 expression in Luminal (ER+) or HER2+ disease (Figure 5E and Supplementary Table S4C).[11]

Discussion

In this study we have shown that Pin1 is highly expressed in a subset of TNBC and plays an important role in pathogenesis and response to treatment. We show that knockdown of Pin1 in TNBC cell lines results in cell death, while increased proliferation is observed in normal breast cell lines. Pin1 functions as a differential modulator of chemotherapy response with both BRCA1-depdendent and independent roles. In the context of the antimicrotubule agent Taxol, knockdown of Pin1 results in increased sensitivity but only in the absence of functional BRCA1. We suggest this is mediated through Pin1-dependent stabilization of Mcl-1. Conversely, knockdown of Pin1 results in decreased sensitivity to DNA-damaging chemotherapy. This is observed in both BRCA1 proficient and deficient cell lines. This translates to a potential role for Pin1 as a biomarker to predict response to DNA damaging chemotherapy, which is SoC for TNBC (Figure 6).

Pin1 has been shown to be essential for breast development and plays an oncogenic role in a number of cancer types including breast cancer.[15,26] Pin1 functions as a prolyl isomerase, catalysing the cis-trans isomerization of proline residues found within pSer/Thr-Pro motifs. This Pin1-dependent isomerization regulates the conformation, and thus the function, of many key proteins, which impacts on many cellular pathways implicated in cancer, including ER-α, NFκB, Stat3, β-catenin, CyclinD1, AKT and Notch.[27] This highlights the potential of Pin1 as a target for therapy. This is further supported by the differential effect of loss of Pin1 expression in cancer *versus* normal cells observed in our study, indicating cancer specificity. However, the increase in cell proliferation observed in normal cells also supports the potential role of Pin1 as a 'conditional tumour suppressor' as loss of Pin1 is associated with increased expression of cell cycle proteins (e.g. Cyclin E and D) and oncogenes (e.g. MYC) in specific genetic backgrounds.[28] Together with our result, this highlights the continuing need to understand the function of Pin1 in health and disease.

While a number of previous studies have shown that inhibition of Pin1 can sensitize cells to various chemotherapies,[29–32] this is the first time that Pin1 has been shown to differentially modulate the response to Taxanes and DNA-damaging chemotherapy in a BRCA1 dependent and independent manner, respectively. It is important to

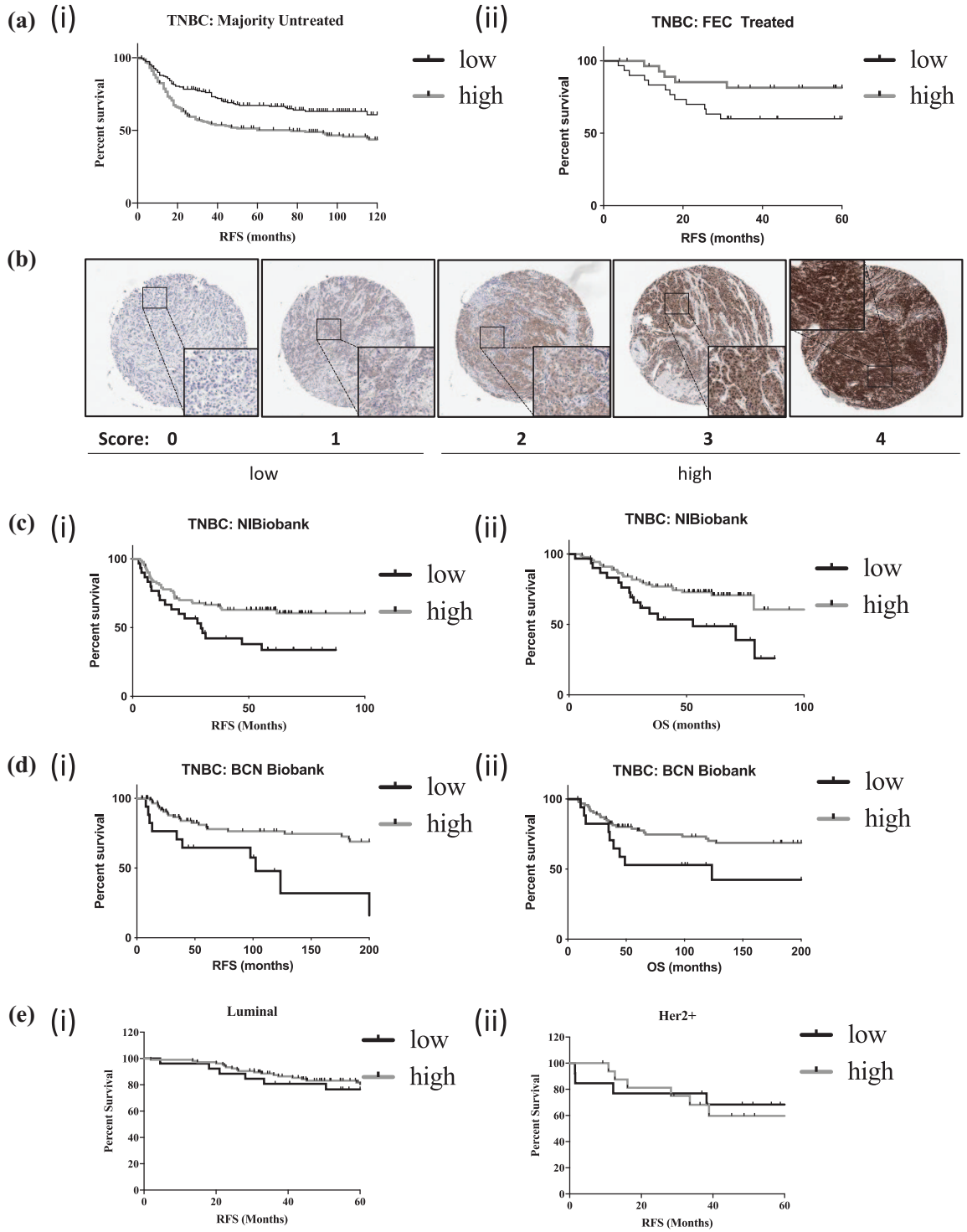

Figure 5. (a) Kaplan–Meier curve of RFS in two TNBC datasets representing majority untreated GSE31519 (i) and FEC-treated patients (ii)[9] stratified based on high (above median) or low (below median) Pin1 gene expression. (b) Representative images at ×10 magnification (×40 for inset) demonstrating the scoring system from 0 to 4 IHC-assessed Pin1 expression. Kaplan–Meier curve of RFS (i) or OS (ii) in the (c) Northern Ireland Biobank or (d) BCN Biobank TNBC TMA cohorts stratified based on low (0 and 1) or high [2–4] IHC-assessed Pin1 expression. (e) Kaplan–Meier curve of RFS in Luminal (ER+/HER2–) and HER2+ (ER–/HER2+) patients from the NIB Breast 300 cohort stratified based on low (0 and 1) or high [2–4] IHC-assessed Pin1 expression. All HRs, 95% CIs and *p* values are reported in Supplementary Table S4.

BCN, Breast Cancer Now; CI, confidence interval; FEC, 5-FU, Epirubicin and Cyclophosphamide; HR, hazard ratio; IHC, immunohistochemistry; NIB, Northern Ireland Biobank; OS, overall survival; RFS, relapse-free survival; TMA, tissue microarray; TNBC, triple negative breast cancer.

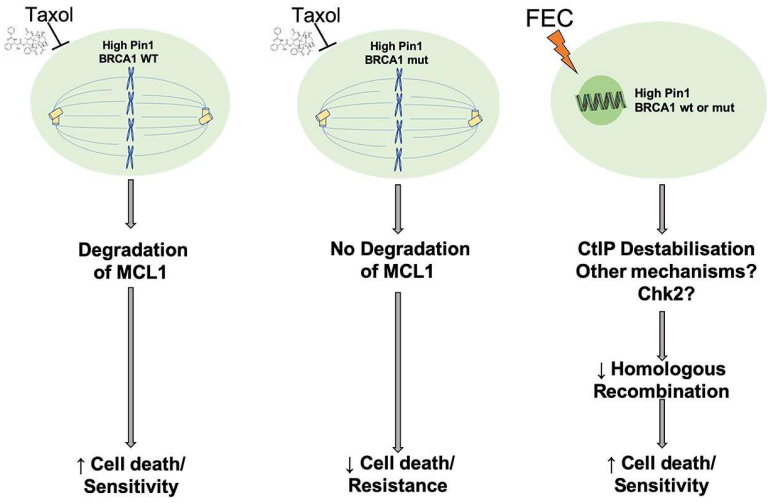

Figure 6. Schematic summarizing the role of Pin1 in modulating response to chemotherapy in TNBC.
FEC, 5-FU, Epirubicin and Cyclophosphamide; mut, mutated; TNBC, triple negative breast cancer; WT, wild type.

note that while Taxanes primarily function through inhibiting mitosis, DNA damage may also be caused as a consequence of mitotic catastrophe. Therefore, these differential effects may not be simply attributed to the primary mechanism of action of these drugs but may also be influenced by differential Pin1 isomerization of proteins involved in drug metabolism, export or DNA damage specific to each drug type.

Our results support the findings of Ding and colleagues, who also demonstrated that Mcl-1 plays a crucial role downstream of Pin1 in resistance to Taxol.[32] However, the BRCA1-dependent phenotype was not described. This indicates that this may be TNBC specific as the cells used in the previous study were either ER+ (MCF-7) or since shown to orginate from melanoma (MDA-MB-435).[33] This supports the tissue-specific role of BRCA1 as recently highlighted by Jonsson and colleagues.[34] Our results indicate a novel therapeutic strategy to resensitize BRCA1 mutant/dysfunction TNBC tumours to Taxol using either direct inhibitors of Mcl-1 or potentially indirect regulators such as Sorafenib as suggested by Ding and colleagues.[32]

In contrast, the CtIP-dependent mechanism underpinning the BRCA1-independent role of Pin1 in conferring sensitivity to DNA damaging agents reported by Steger and colleagues could not be recapitulated in our breast cell lines.[20] This further highlights the context specific role of Pin1, whereby it differentially modulates the response to different cellular stresses. This is supported by the recent publication from the Morris laboratory demonstrating that, in the context of replication stress, Pin1 enhances the interaction between BRCA1-BARD1 and RAD51, increasing the presence of RAD51 at stalled replication forks and therefore promoting fork protection.[35] Our findings also highlight the fact that Pin1 may modulate the same response through different mechanisms in different cancer types. Unravelling the exact mechanism by which Pin1 regulates response to DNA damaging agents was beyond the scope of the current project, but through preliminary analysis of a phosphokinase array used to identify key pathways regulated by Pin1,[5] we have identified and validated the DNA repair protein CHK2 as a novel Pin1 target gene (Supplementary Figure S4A). Knockdown of Pin1 results in loss of phosphorylation of tyrosine 68, a key site in the activation of the kinase, indicating this may be a mechanism of resistance that warrants further investigation.

Regardless of the mechanism by which Pin1 regulates response to DNA-damaging chemotherapy, this study highlights the novel potential role of Pin1 as a biomarker to identify women likely to receive clinical benefit from current SoC chemotherapy regimens such as FEC. Furthermore, preliminary analysis of cell line and patient data indicates this may be extended to Cisplatin and Parp inhibitors, which are key treatment options emerging for the management of TNBC.[4] Using publicly available data, we have shown that Pin1 gene expression negatively correlates with IC_{50} concentrations of Cisplatin (Supplementary Figure S4B) and the Parp inhibitor, Rucaparib (Supplementary Figure S4C), in TNBC cell lines but not breast cancer cell

lines as a whole (Supplementary Table S5A).[36] We have recapitulated this in vitro, demonstrating that knockdown of Pin1 results in decreased sensitivity to both agents in the MDA-MB-468 cell line (Supplementary Figure S3D and Supplementary Table S5B). Furthermore, analysis of TNBC patients treated with Cisplatin shows that Pin1 expression correlates significantly ($p < 0.0001$) with treatment response quantified using the Miller-Payne scale (Supplementary Table S4C).[37] Based on our own results in the context of SoC, and the preliminary indications in the context of other treatment, this warrants further analysis of the potential role of Pin1 as a prognostic and predictive biomarker in additional patient cohorts.

In conclusion, this study demonstrates the oncogenic role of Pin1 driving treatment response in TNBC. The ability of Pin1 to differentially modulate response to treatment emphasizes its context-specific function and highlights the need for continued investigations into the roles of Pin1 in normal cell function as well as cancer. Finally, we highlight the potential of Pin1 as a novel biomarker for the stratification of TNBC patients for treatment in order to improve the management of this poor outcome cancer.

Acknowledgements

The authors also wish to acknowledge the role of the Breast Cancer Now Tissue Bank in collecting and making available the samples used in the generation of this publication.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and publication of this article: This work was funded by Breast Cancer Now (2012MaySF112 and 2013NovPhD184)

Samples used in this research were received from the Northern Ireland Biobank (NIB), which is funded by the Health and Social Care (HSC) Research and Development Division of the Public Health Agency in Northern Ireland and Cancer Research UK through the Belfast Cancer Research UK (CRUK) Centre and the Northern Ireland Experimental Cancer Medicine Centre; additional support was received from the Friends of the Cancer Centre. The Northern Ireland Molecular Pathology Laboratory, which is responsible for creating resources for the NIB has received funding from CRUK, the Friends of the Cancer Centre and the Sean Crummey Foundation.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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