Research article

**Estrogen receptor alpha (ESR1)-signaling regulates the expression of the taxane-response biomarker PRP4K**

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**ABSTRACT**

The pre-mRNA splicing factor 4 kinase PRP4K (PRPF4B), is an essential kinase that is a component of the U5 snRNP and functions in spliceosome assembly. We demonstrated that PRP4K is a novel biological marker for taxane response in ovarian cancer patients and reduced levels of PRP4K correlate with intrinsic and acquired taxane resistance in both breast and ovarian cancer. Breast cancer treatments are chosen based on hormone and growth factor receptor status, with HER2 (ERBB2) positive breast cancer patients receiving anti-HER2 agents and taxanes and estrogen receptor alpha (ESR1) positive (ER+) breast cancer patients receiving anti-estrogen therapies such as tamoxifen. Here we demonstrate that PRP4K is expressed in the normal mammary duct epithelial cells of the mouse, and that estrogen induces PRP4K gene and protein expression in ER+ human MCF7 breast cancer cells. Estrogen acts through ESR1 to regulate PRP4K expression, as over-expression of ESR1 in the ER-negative MDA-MB-231 breast cancer cell line increased the expression of this kinase, and knock-down of ESR1 in ER+ T47D breast cancer cells reduced PRP4K levels. Furthermore, treatment with 4-hydroxytamoxifen (4-OHT) resulted in a dose-dependent decrease in PRP4K protein expression in MCF7 cells. Consistent with our previous studies identifying PRP4K as a taxane-response biomarker, reduced PRP4K expression in 4-OHT-treated cells correlated with reduced sensitivity to paclitaxel. Thus, PRP4K is novel estrogen regulated kinase, and its levels can be reduced by 4-OHT in ER+ breast cancer cells altering their response to taxanes.

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**1. Introduction**

Breast cancer, the most common malignancy in women, is a hormone-dependent cancer that responds to the sex steroid hormone estrogen. Although there are multiple forms of estrogen, including estrone (E1) and estriol (E3), the most predominant and potent human estrogen is 17β-estradiol (E2) [1]. Estrogen is implicated in a wide variety of physiological functions including the regulation of both cell proliferation and differentiation of mammary glands, uterine endometrium and ovarian cells [2], while also providing a mechanism through which the growth of hormone-related cancers are regulated [3]. Estrogen exerts its effect via binding to one of the two estrogen receptors (ERs), ERα (ESR1) or ERβ (ESR2), which are members of the super-family of nuclear receptors [4]. In response to estrogen-binding, these receptors act as ligand activated transcription factors and many estrogen regulated genes have been shown to play a role in breast cancer cell proliferation, including the oncoproteins MYC and cyclin D1 [5]. Breast cancer cells express a number of other hormone and growth factor receptors in addition to ERα, including the progesterone receptor (PR) and the human epiderminal growth factor receptor HER2 (ERBB2). The receptor status of the breast cancer serves as both a prognostic marker of outcome as well as a predictive marker for response to targeted therapies [6–8]. For example, HER2 positive breast cancer patients typically have a poor prognosis but respond to combination therapies of an anti-HER2 agent such as the anti-HER2 antibody trastazumab (Herceptin) with microtubule poisons such as taxanes. On the other hand, ER+ breast cancers have a much better prognosis and respond well to anti-estrogen therapies such as tamoxifen which inhibits the estrogen receptor directly, fulvestrant which down-regulates estrogen receptor expression, or by inhibiting estrogen synthesis using aromatase inhibitors [9,10]. Patients with ER+ breast cancer are not typically treated with taxanes as a first-line therapy alone or in combination with other chemotherapy, as taxanes provided little additional benefit for these patients in clinical trials [11]. However, a significant number of ER+ breast cancer patients have breast cancers that are resistant to a given anti-estrogen therapy de novo
or relapse during treatment, presumably due to acquired resistance, and as a result are treated with other anti-hormonal agents and/or additional chemotherapy including taxanes [12,13]. Thus, biomarkers for taxane response could prove very useful in this context to stratify patients into potential responders and non-responders to taxane treatment [14].

Recently, we identified the pre-mRNA splicing factor 4 kinase (PRP4K), also known as PRPF48, as a novel Her2-regulated biomarker of taxane response in breast and ovarian cancer [15]. PRP4K was first characterized in a temperature-sensitive mutant screen for genes affecting pre-mRNA splicing in *Schizosaccharomyces pombe* [16]. At the restrictive temperature, fission yeast carrying a temperature sensitive mutation in prp4 accumulated un-spliced pre-mRNA. Subsequent characterization of the prp4 gene revealed that the splicing factor encoded a serine/threonine kinase [17]. We demonstrated that the mammalian homolog of prp4 (PRP4K) can interact with pre-mRNA splicing factors PRP6 and Suppressor-of-White-Apricot (SWAP) and can be co-purified with the US snRNP [18]. Later PRP4K was found to be a key regulator of spliceosomal assembly through the phosphorylation of PRP6 and PRP31 [18,19]. PRP4K has also been implicated in regulation of the spindle-assembly checkpoint (SAC) [20], which may explain how reduced levels of PRP4K contribute to resistance to microtubule poisons like the taxane paclitaxel [15]. Here we demonstrate that PRP4K is a novel estrogen-regulated kinase and that manipulation of estrogen signalling can alter PRP4K levels, which in turn can impact taxane sensitivity in ER+ breast cancer cells.

### 2. Methods

#### 2.1. Cell culture, drug and hormone treatment

MCF7, T47D and MDA-MB-231 cells were purchased from American Type Culture Collection (ATCC) and validated by STR profiling (DCC Medical). Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (Sigma) supplemented with 10% Fetal Bovine Serum (FBS) (Invitroge, Canada) and 1% penicillin/ streptomycin at 37 °C with 5% CO2. For estradiol experiments cells were cultured in phenol red free DMEM (Sigma) supplemented with 5% carbon- stripped FBS (Life Technology) for 48 h prior to the addition of 10 nM or 100 nM 17-β-estradiol (E2) (Sigma) or vehicle control (0.01% ETOH). Cells were harvested 24 h post-treatment for either protein lysate extraction or total RNA extraction. For tamoxifen experiments cells were cultured in phenol red free DMEM (Sigma) supplemented with 5% carbon- stripped FBS and 10 nM E2 for 24 h prior to being treated with the indicated dose of 4-hydroxytamoxifen (4-OHT), or vehicle (0.05% DMSO) for 24 h.

#### 2.2. Indirect immunofluorescence detection of PRP4K in murine mammary tissue

Mouse mammary glands were dissected from 3 month old virgin mice and mammary tissue was fixed in 4% paraformaldehyde (PFA) at 4 °C, paraffin-embedded, sectioned (at 5 μm), and stained with hematoxylin and eosin. For the immunofluorescence detection of PRP4K, sheep anti-PRP4K H143 antibody (1:200) [15,18] was incubated with (control) or with a blocking re-combinant peptide (1 μg/ml) (504 to 688 aa of human PRP4K) before being used for the immunodetection of PRP4K on tissue sections, followed by incubation with a donkey anti-goat Cy3 secondary antibody (1:500)[Jackson Immunoresearch]. Tissue slides where then imaged on a Nikon Eclipse E600 equipped with a Nikon DXM1200F CCD camera using the software ACT-1.

#### 2.3. Western blotting and densitometry

Cells were harvested and lysed in ice-cold lysis buffer (300 nM KCl, 20 mM Tris–HCl pH 8, 10% Glycerol, 0.25% Nonidet P-40, 0.5 mM EGTA, 0.5 mM EDTA, 1X protease inhibitors). Total protein concentrations were quantified using Bio-Rad protein reagent (Bio-Rad) and 1% BSA (Bio-Rad) to generate a standard curve. Lysates were mixed with 2X sample buffer and boiled at 95 °C for 5 min prior to separation by SDS-PAGE and Western blot analysis with sheep anti-PRP4K (H143) [18], rabbit anti-ESR1 (Cell Signaling), mouse anti-actin (Sigma) or rabbit anti-tubulin (Sigma) antibodies prior to protein detection by enhanced chemiluminescence (ECL) (Bio Rad). Chemiluminescence was then visualized either by exposing radiographic film (Kodak) or using a VersaDoc imaging system MP4000 (Bio Rad). Quantification of band intensities was performed by densitometry analysis using the QuantityOne software (Biorad; version 4.6.8) or with ImageJ (NIH; version 1.49), with at least three separate experiments used to determine mean fold changes in protein expression.

#### 2.4. RNA isolation and quantitative real-time PCR (q-RTPCR)

TRizol Reagent (Invitrogen) was used to lyse cultured cells and total RNA was extracted according to the manufacturer’s protocol. RNA was quantified using a Biophotometer (Eppendorf). One microgram of RNA was converted to cDNA using the Quantitect Reverse Transcription kit (Qiagen), employing universal hexamer primers and reverse transcriptase according to manufacturer’s protocol. Quantitative PCR (Q-PCR) was then performed on the cDNA using a Quantitect primer assay specific to PRP4F4B and RPLP0 (endogenous control gene) (Hs_RPLP0_2_5G and Hs_PRP4F4B_1_5G) (Qiagen). Q-PCR reactions were performed using a Maxpro3000 real-time PCR detection system (Stratagene) under the standard real-time cycle conditions suggested by the manufacturer. The relative expression levels were then calculated between experimental treatments by normalizing PRP4F4B to RPLP0 expression using the ΔΔCt method [21]. Three technical replicates were done per experiment for each condition, and all experiments were performed in triplicate.

#### 2.5. Cell transfection and shRNA Lentiviral Transduction

For ESR1 transfections, MDA-MB-231 cells were transfected with the ESR1 expression vector pcDNA-HA-ER WT (a gift from Sarat Chandrarlapaty (Addgene plasmid # 49498)) using Lipofectamine 2000 according to the manufactures protocol. After 12–18 h cells were harvested for protein lysate preparation and western blot analysis was performed as described above.

To knockdown ESR1 in the T47D cell line, ESR1 targeting GIPZ Lentiviral shRNAs (shESR1-1-clone: V2LH5_239590, shESR1-2-clone: V2LH5_239351) were purchased from Thermos Scientific. Lentivirus was obtained by co-transfection of the TRIPZ shRNA, pMD2.G, pCMV-8.92, and pCMV-8.93 vectors (described previously [22]) into human HEK-293T cells via calcium-phosphate transfection (Promega), according to the manufacturer’s protocol. After 48 h, media from the transfected cells was filter sterilized using a 0.45 μ filter, and the viral media added to T47D cells for 48 h, after which the media was exchanged for media supplemented with 2 μg/ml of puromycin and positively transduced cells were selected in the presence of antibiotic for 72 h prior to further experimentation.

#### 2.6. Taxane treatment and cell viability assay

Paclitaxel (Sigma) and 4-OHT (Sigma) were reconstituted in
dimethyl sulfoxide (DMSO) and diluted in growth media so that the DMSO concentration was 0.05% or less. To evaluate MCF7 cell response to combined doses of tamoxifen and taxane, 5,000 cells were plated in individual wells of a 96 well plate, and allowed to adhere for 24 h in phenol red free DMEM (Sigma) supplemented with 5% carbon- stripped FBS (Life Technology) supplemented with 10 nM 17β-estradiol (E2) prior to incubation with 10 μM 4-OHT or vehicle for an additional 24 h. After 24 h of 4-OHT treatment, the drug was removed and media was replaced by media containing the indicated doses of paclitaxel for 90 min as previously described [15]. Following acute paclitaxel treatment, the drug was removed and cells were allowed to recover in fresh medium for 72 h, at which point cell viability was measured using the AlamarBlue cell viability assay (Life Technologies), according to the manufacturers protocol. Consequently, viability was measured by monitoring fluorescence using a SpectraMax M2 plate reader (Molecular Devices) 4 h after the addition of AlamarBlue reagent. Values were normalized to DMSO-treated cells, and derived from at least three technical replicates. All experiments were repeated in triplicate.

2.7. Statistical analysis

For comparison of PRP4K expression by Western blot and Q-PCR in cells treated with E2, or differences in viability by AlamarBlue assay, Student’s T-test was used to estimate the significance of measured values between treatments using Excel (Microsoft). For comparisons of PRP4K (PRPF4B) expression changes in the gene array experiments found in references [23–25], we identified the corresponding data sets in the GEO profiles database [26] from the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/). Using the GEO profile data, normalized log-transformed expression counts (for at least 2 separate array probes specific for PRPF4B) were combined for control treated and E2 treated cell replicates to determine average fold change for a given dataset, and a Student's T-test was used to provide an estimate of significance using Excel.

3. Results

3.1. PRP4K is expressed in mammary duct epithelial cells and is regulated by 17β-estradiol via the estrogen receptor alpha (ESR1)

The pre-mRNA splicing kinase PRP4K is an essential kinase that is ubiquitously expressed in the brain and hematopoietic cells [18,27]. Unpublished observations of the immunohistochemical detection of PRP4K available from the Protein Atlas web resource (http://www.proteinatlas.org/) indicated that PRP4K may also be highly expressed in the epithelial cells lining the ducts of the ovary and breast. Using indirect immunofluorescence detection of PRP4K in murine mammary tissue, we confirmed that PRP4K is predominately expressed in the epithelial layer of the ducts (Fig. 1). These epithelial cells of the milk duct express the estrogen receptor-alpha (ERα/ESR1), suggesting that perhaps PRP4K protein expression may be regulated by estrogen signaling via this receptor. To lend support to this hypothesis, we analysed 3 publicly available gene expression data sets in which either osteosarcoma cells expressing ERα or MCF7 ER+ breast cancer cells were treated with 10–25 nM 17β-estradiol (E2) [23,25]. Although the Bourdeau et al., study did not reveal a significant increase in PRP4K (PRPF4B) gene expression, two of these studies Lin et al., and Stossi et al., did show a significant increase in PRPF4B transcript levels in response to E2 treatment. The greatest increase was seen in the study by Stossi and colleagues, where treatment with 10 nM E2 for 24 h resulted in ~2-fold (p < 0.02) increase in PRPF4B transcript levels in osteosarcoma cells ectopically expressing ERα [23].

To confirm that PRP4K expression is indeed regulated by estrogen, we treated ER+ MCF7 breast cancer cells with vehicle (0.01% ETOH) or with E2. After 24 h of exposure to 10 nM E2, we observed a 2-3 fold increase in PRP4K transcription as measured by quantity PCR (Q-PCR) (2.7 ± 0.6)(p < 0.05)(Fig. 2A). Similarly, both 10 and 100 nM E2 treatment resulted in an increase in protein expression of 2-2.5 fold (1.8 ± 0.2 and 2.2 ± 0.4, respectively) (p < 0.05)(Fig. 2B and C). To determine how rapid PRP4K transcript levels were increasing after estrogen treatment, we also carried out a time course measuring PRP4K levels by Q-PCR at 0.5 h, 6 h and 12 h posttreatment with 10 nM E2 (Supplemental Fig. S1). We observed a significant induction of PRP4K transcript levels of ~1.4 fold (p < 0.05) as early 0.5 h post-treatment with E2, followed by a drop in transcript and a second peak in gene expression at 12 h (~2.7 fold; p < 0.05). However, we did not see a significant protein accumulation until 24 h post-treatment with E2 (data not shown).

To investigate if PRP4K gene regulation by estrogen was dependent on ERα/ESR1, ERα expression was knocked-down in the ER+ T47D breast cancer cell line using small hairpin-mediated RNA interference (shRNA) directed against ESR1. Consistent with PRP4K being regulated by estrogen via ERα, ESR1 knock-down correlated with decreased levels of PRP4K protein (Fig. 3A). Conversely, overexpression of ERα in the ER-negative breast cancer cell line MDA-MB-231 resulted in increased PRP4K protein levels (Fig. 3B). Thus, these data indicate that PRP4K is a novel estrogen-regulated kinase expressed in normal mammary epithelium and in breast cancer cells, and whose expression can be regulated through ERα signalling.

![Fig. 1. PRP4K is expressed in mammary duct epithelial cells. A-B] Murine mammary tissue was harvest, fixed, embedded in paraffin and processed for immunohistochemical analysis of PRP4K localization by indirect immunofluorescence (A) with (anti-PRP4K; right panel) or without primary antibody (no primary; left panel), and general tissue morphology by hematoxylin and eosin (H&E) staining (B). Milk ducts lined with mammary epithelial cells are indicated by white arrows.
administration of fluoropyrimidine plus tamoxifen or tamoxifen plus anthracyclines and/or taxanes has been used to treat both ER+ and ER- patients [32–35]. However, anti-hormone treatments can also negatively impact the efficacy of chemotherapy. In particular, several clinical studies have reported antagonistic effects of tamoxifen on the efficacy of chemotherapeutic agents based on timing of administration [33,36]. The exact mechanism leading to the synergistic or antagonistic effects of anti-hormone therapies with these chemotherapeutic agents has yet to be fully understood.

We previously demonstrated that low PRP4K expression is associated with intrinsic and acquired taxane resistance in breast and ovarian cancer [15]. Thus, changes in PRP4K protein levels mediated by estrogen signalling could conceivably alter the taxane response of ER+ breast cancer cells. To address this possibility, we first determined the impact of tamoxifen on PRP4K protein levels in ER+ MCF7 breast cancer cells (Fig. 4). Treatment of MCF7 with 4-hydroxytamoxifen (4-OHT), the active metabolite of tamoxifen, in defined media containing 10 nM E2 resulted in a dose-dependent decrease in PRP4K protein levels with up to a 80% reduction in PRP4K protein at a dose of 10 μM (Fig. 4A), which correlated with a 28% reduction in PRP4K transcript levels (i.e. 0.72 ± 0.08 fold change compared to control; p < 0.05) (Fig. 4B).

Since a concentration of 10 μM 4-OHT robustly inhibited PRP4K expression, we sought to determine if tamoxifen treatment could alter the taxane response of ER+ breast cancer cells. To this end, ER+ MCF7 breast cancer cells were sequentially treated with 10 μM 4-OHT in defined media containing 10 nM E2, followed by various concentrations of the taxane paclitaxel. Resistance to a range of doses of paclitaxel (from 0.3 to 1.25 μM) was significantly increased in cells pretreated with tamoxifen as compared to vehicle-treated cells (Fig. 5). This data indicates that inhibition of estrogen receptor signalling by 4-OHT results in a

![Fig. 2. Induction of PRP4K gene and protein expression by 17-β-estradiol. A) MCF7 cells were treated for 24 h with either vehicle (0.01% ETOH) (Ctrl) or 10 nM 17-β-estradiol (E2) before RNA was extracted for Q-PCR to determine the relative levels of PRP4K gene expression in response to estrogen as indicated in the bar graph. Error bars = SEM (where n=3); p < 0.05. B) MCF7 cells were treated for 24 h with either vehicle (0.01% ETOH) (Ctrl) or 17-β-estradiol (E2) at the indicated concentration before being processed for Western blot and densitometry analysis. A representative Western blot is shown in (B) and a bar graph of the relative fold change in PRP4K protein level relative to actin after 24 h treatment with E2 is shown in (C). Error bars = SEM (where n=4); p < 0.05.

![Fig. 3. Knock-down or over-expression of ESR1 alters PRP4K gene expression. A) Western blot analysis of PRP4K and ESR1 levels in ER+ breast cancer cell line T47D following transduction with retrovirus carrying a short-hairpin RNA (shRNA) (Ctrl) or one of two different shRNAs directed against the ESR1 mRNA (shRNA-ESR1-1 and -2, respectively). Actin was used as a loading control and the ratio of PRP4K in control cells normalized to actin is shown. B) Western blot analysis of PRP4K and ESR1 levels in ER- breast cancer cell line MDA-MB-231 following transduction with an empty vector (Ctrl) or a vector carrying the human ESR1 gene (+ESR1). Actin was used as a loading control and the ratio of PRP4K in control cells normalized to tubulin is shown.

3.2. 4-Hydroxytamoxifen treatment down-regulates PRP4K expression and modifies taxane response in MCF7 breast cancer cells

Patients with ER+ positive breast cancers have a better prognosis compared to those with ER-negative breast cancers [28–30]. This is in part due to the availability of anti-estrogen agents such as tamoxifen that binds and inhibits ERα, leading to inhibition of ERα breast cancer growth [31]. Anti-estrogen treatments are typically combined with other treatments. For example, combined

![Fig. 4. Tamoxifen reduces PRP4K gene expression. A-B) Western blot analysis of PRP4K in ER+ MCF7 breast cancer cells following 24 h treatment with vehicle (0.05% DMSO) (Ctrl) or increasing concentrations of 4-hydroxytamoxifen (4-OHT) as indicated. Actin was used as a loading control and the ratio of PRP4K in control cells normalized to actin is shown. B) Q-PCR analysis of PRP4K gene expression in MCF7 cells treated with vehicle (0.05% DMSO)(Ctrl) or 10 μM 4-OHT is shown as a bar graph. Error bars = SEM (where n=3); p < 0.05.

Please cite this article as: S. Lahsaee, et al., Estrogen receptor alpha (ESR1)-signaling regulates the expression of the taxane-response biomarker PRP4K, Exp Cell Res (2015), http://dx.doi.org/10.1016/j.yexcr.2015.12.013
need to be treated with other hormonal therapies and/or additional systemic chemotherapy, including treatment with taxanes. Taxanes have significant side-effects, including peripheral neuropathy, cardiotoxicity and neutropenia, which greatly affect quality of life [48,49]. Thus, there is growing interest in finding biomarkers that predict treatment response to taxanes not only during initial treatment but also post-relapse to prevent unnecessary treatment in potentially non-responsive patients.

We recently characterized PRP4 kinase (PRP4K, also known as PRPF4B) as a novel HER2-regulated modifier of taxane response in breast and ovarian cancer [15]. Not only is PRP4K expressed in breast cancer, we demonstrate here that PRP4K is expressed in normal mammary epithelial cells as well (Fig. 1), in agreement with publicly available tissue microarray data from the Human Protein Atlas [50]. Given the localization and expression of PRP4K in mammary epithelial cells, which express ERα, we sought to determine if PRP4K could be regulated by estrogen-signalling. Our Q-PCR and Western blot analysis confirmed that upon treatment of ER+ MCF7 breast cancer cells with 17β-estradiol, PRP4K protein and transcript levels increased ~2-fold (Fig. 2). Furthermore, knock-down of ERα/ESR1 in ER+ T47D breast cancer cells, or over-expression of ERα in triple negative MDA-MB-231 cells, correlated with a decrease or increase of PRP4K protein expression (respectively), suggesting that PRP4K levels are regulated by estrogen-signalling via ERα (Fig. 3). Regulation by ERα-dependent signalling was further confirmed by treatment of cells with 4-hydroxymethoxifen (4-OHT) in the presence of 10 nM E2, which reduced PRP4K protein expression in a dose-dependent manner (Fig. 4).

Based on the Q-PCR data, PRP4K appears to be regulated transcriptionally by estrogen. Surprisingly, we could not find estrogen response elements (ERE) by sequence analysis of the PRPF4B promoter region, or direct binding of ERα in the ChIP-seq data found for ER+ MCF7 cells in the ENCODE dataset [51] available through the University of California at San Diego (UCSD) Genome Browser (https://genome.ucsc.edu/). However, we did find evidence of Sp1 and MYC binding sites in the ChIP-seq data, which are known to either cooperate or to be regulated by ERs themselves [5,52] and thus could modulate PRP4B expression in response to estrogen. Finally, we also demonstrated in this study that a reduction in PRP4K protein levels, induced by treatment of MCF7 breast cancer cells with 4-OHT, correlates with reduced sensitivity of these cells to taxanes (Fig. 5).

Previous clinical studies have shown that sequential use of tamoxifen and anthracycline/cyclophosphamide chemotherapy is more efficient than concurrent use of these drugs [33]. These studies suggest that differential sequencing or timing of tamoxifen with chemotherapy is an important factor in improving treatment response. Moreover, previous in vitro studies have reported that anti-hormone drugs can have an antagonistic effect on some chemotherapy regimens [36,53]. Increased levels of MAPT/tau, BCL2 and p-glycoprotein (MRP1) are associated, in vitro, with taxane sensitivity [54]. Although the mechanism of tamoxifen antagonistic action with taxanes is not very well understood, Ikeda and colleagues demonstrated a possible role for BCL2 and MAPT, whose expression increased slightly in tamoxifen-treated cells and these changes in expression correlated with decreased sensitivity to taxanes [55]. However, they also observed a decrease in MRP1, which is inconsistent with enhanced resistance and thus unlikely to play a role in the antagonistic effects of tamoxifen on the taxane response [55].

In this study, MCF7 cells treated with 4-OHT showed a dose-dependent decrease in PRP4K protein expression (Fig. 4), a modifier of taxane sensitivity as discussed above [15], which correlated with decreases sensitivity of 4-OHT treated cells to the taxane paclitaxel (Fig. 5). These data indicate 4-OHT may modulate the effect of taxanes on ER+ cells in part by reducing PRP4K

Fig. 5. Treatment with tamoxifen reduces the sensitivity of ER+ MCF7 cells to paclitaxel. ER+ MCF7 breast cancer cells grown in carbon-stripped media containing 10 nM E2 were treated for 24 h with either vehicle (0.05% DMSO) or 10 μM 4-hydroxytamoxifen (4-OHT) prior to acute treatment with paclitaxel at increasing doses as indicated for 90 min. Cells were allowed to recover after drug treatment for an additional 24 h prior to measure of viability. Percentage viability is shown versus concentration of paclitaxel for vehicle versus tamoxifen treated cells. Error bars = SEM (where n = 3); **p < 0.02.
protein levels, which would in turn alter spindle-assembly checkpoint activity [20] allowing treated cells to escape cell death by “mitotic-slippage” [15]. Thus in addition to identifying PRP4K as a novel estrogen-regulated kinase, our study provides new insight as to how anti-estrogen treatments may negatively impact the taxane response of ER+ breast cancer. Since taxane resistance is multi-factorial, PRP4K represents only one factor contributing to the possible antagonistic effects of tamoxifen in modulating the taxane response in breast cancer. Thus, in addition to PRP4K other modifiers of taxane drug response including BCL2, MAPT [54] when evaluated together may prove useful in stratifying ER+ breast cancer patients based on potential benefit from taxane therapy. A final corollary to our observations is that patients treated with drugs that inhibit estrogen signalling other than selective estrogen receptor modulators (SERMs) like tamoxifen, including the aromatase inhibitor anastrozole, and the selective estrogen down-regulator (SERD) fulvestrant [9,10], could potentially alter taxane sensitivity through the modulation of PRP4K protein levels. Consequently, future studies should evaluate the optimal combination(s) and sequencing of anti-estrogen therapies with taxanes, as our work suggests pre-treatment with these agents may have a negative impact on the biological response of ER+ breast cancers to these microtubule-targeting drugs.

Acknowledgments

We would like to thank Pat Colp and Colleen Mitchell for their aid in the immunohistochemical detection of PRP4K. This research was funded by an operating grant to GD from the Canadian Breast Cancer Foundation (CBCF) – Atlantic (R14 F09). GD is a Senior Scientist of the Beatrice Hunter Cancer Research Institute (BHCRI). LEA is supported by a studentship from the Canada Graduate Scholarships-Master’s Program (CGSM) at the Natural Sciences and Engineering Research Council (NSERC) of Canada, and DPC was supported by a CIBC Graduate Scholarship in Medical Research awarded to GD by the BC Cancer Research Institute (BHCRI) and The Canadian Cancer Society, Nova Scotia Division as part of the Terry Fox Foundation Strategic Health Research Training (STIHR) Program in Cancer Research at the Canadian Institutes of Health Research (CIHR). DPC was also supported by funds to GD from CiclicGear International Ltd. via the Dalhousie Medical Research Foundation’s “Adopt-a-Researcher” program. Finally, we would like to dedicate this study to Mrs. Kate Kimberley during her own courageous and personal battle against breast cancer.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.yexcr.2015.12.013.

References


