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Proteomic modulation in breast tumors after metformin exposure: results from a “window of opportunity” trial

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Abstract

Purpose—Reverse Phase Protein Array (RPPA) is a high-throughput antibody-based technique to assess cellular protein activity. The goal of this study was to assess protein marker changes by RPPA in tumor tissue from a pre-surgical metformin trial in women with operable breast cancer (BC).

Methods—In an open-label trial, metformin 1500-mg PO daily was administered prior to resection in 35 non-diabetic patients with stage 0–III BC, body mass index ≥ 25 kg/m². For RPPA, formalin-fixed paraffin-embedded (FFPE) samples were probed with 160 antibodies. Paired and two-sample t-tests were performed ($p < 0.05$). Multiple comparisons were adjusted for by fixing

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Informed consent Informed consent was obtained from all individual participants included in the study.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Conflict of interest There are no conflicts of interest to declare for this study.

the false discovery rate at 25 %. We evaluated whether pre- and post-metformin changes of select markers by RPPA were identified by immunohistochemistry (IHC) in these samples. We also assessed for these changes by western blot in metformin-treated BC cell lines.

Results—After adjusting for multiple comparisons in the 32 tumors from metformin-treated patients vs. 34 untreated historical controls, 11 proteins were significantly different between cases vs. controls: increases in Raptor, C-Raf, Cyclin B1, Cyclin D1, TRFC, and Syk; and reductions in pMAPK^{pT202,Y204}, JNK^{pT183,pT185}, Bad^{pS112}, PKC.alpha^{pS657}, and Src^{pY416}. Cyclin D1 change after metformin by IHC was not observed. In cell lines, reductions in JNK^{pT183} and Bad^{pS112} were seen, with no change in Cyclin D1 or Raptor.

Conclusions—These results suggest that metformin modulates apoptosis/cell cycle, cell signaling, and invasion/motility. These findings should be assessed in larger metformin trials. If confirmed, associations between these changes and BC clinical outcome should be evaluated.

Clinicaltrials.gov identifier—.

Keywords

Pre-surgical; Metformin; Reverse Phase Protein Array; Immunohistochemistry; Breast Cancer; Cyclin D1

Introduction

There remains significant interest in the anti-diabetic drug metformin as an anti-breast cancer therapeutic agent [1–8]. Proposed mechanisms of metformin's anti-neoplastic effects include reduction of serum insulin levels, direct modulation of cellular protein synthesis, and growth inhibition through effects in the PI3K/AKT/AMPK signaling pathway [9]. To further characterize the biologic impact of metformin in patients with operable breast cancer, four pre-surgical metformin trials in women with breast cancer have been reported [10–13]. In a pre-surgical or “window of opportunity trial,” patients with newly diagnosed cancer are administered a drug for a limited time period between the time of diagnostic biopsy and surgical excision [14]. The primary endpoint of these studies is biologic, evaluating changes in tumor and/or blood collected before and after administration of the agent.

Mixed results were identified in the pre-surgical metformin trials. Two studies demonstrated no change in tumor proliferation in the overall study cohort [10, 11], as measured by ki-67 immunohistochemistry (IHC) expression, and two studies reported a significant reduction in ki-67 [12, 13]. In our “window of opportunity” trial of metformin in 35 overweight or obese patients with newly diagnosed breast cancer, we did not observe a change in tumor proliferation; however, we identified a significant reduction in other parameters, including body mass index (BMI), serum cholesterol, and serum leptin after an approximately 2-week intervention [10]. While change in pre-surgical ki-67 is a well-validated marker of clinical outcome in patients receiving anti-estrogen therapy [15–18], metformin is a non-hormonal agent that could potentially result in changes in other tumor-based functional proteomic markers. The goal of this study was to identify additional mechanisms by which metformin biologically impacts breast tumor tissue in patients with newly diagnosed breast cancer and to validate these changes by different protein expression assays.

Materials and methods

Clinical trial

Between October 2009 and August 2011, 35 women were enrolled to a single-arm, pre-surgical trial with metformin at the Columbia University Medical Center (CUMC) in New York, NY, as previously described [10]. Patients were administered metformin 1500-mg daily for at least 2 weeks prior to breast surgery. Trial participants were non-diabetic women with a BMI ≥ 25 kg/m², and a recent diagnosis of histologically confirmed stage I–III invasive breast cancer ($n = 25$) or ductal carcinoma in situ (DCIS) ($n = 10$) on core breast biopsy. No prior chemotherapy, radiation therapy, or surgery within 6 months of study entry was allowed. Patients were evaluable if they had sufficient pre- and post-treatment tumor tissue for analysis, and demonstrated at least 75 % compliance with metformin. These cases were matched 1:1 to historical controls by age (± 10 years), stage (TNM), and BMI [± 5 kg/m², and within the same category: overweight (25.0–29.9 kg/m²) or obese (≥ 30.0 kg/m²)] from the Tumor Bank at CUMC. As previously mentioned, the controls were not treated with metformin and were selected from the existing Herbert Irving Comprehensive Cancer center breast database. The institutional review board (IRB) at CUMC approved the study (clinicaltrials.gov identifier:), and all patients included in the study provided informed consent for participation.

Biomarker assessment

Reverse Phase Protein Array (RPPA)

Reverse Phase Protein Array (RPPA) was performed at the M.D. Anderson Cancer Center Functional Proteomics RPPA Core Facility, as previously described [19–22]. Protein extracts from formalin-fixed paraffin-embedded (FFPE) tumor blocks (ten 4- μ m untreated slides) from pre-metformin cores, and post-metformin surgical resection tissue were prepared from appropriately isolated regions (>70 % tumor cells, as determined by an experienced pathologist [HH]). Following deparaffinization, at least 30–40 mg of protein per sample was extracted using an extraction buffer of 20-mM Tris HCl buffer at pH 9, with 2 % (w/v) SDS [23]. RPPA was conducted using a total of 160 antibodies (www.mdanderson.org/education-and-research/resources-for-professionals/scientific-resources/core-facilities-and-services/functional-proteomics-rppa-core/index.html), including markers that we classified as associated with PI3K/AKT/AMPK pathway signaling ($n = 40$), MAPK signaling ($n = 19$), angiogenesis ($n = 6$), apoptosis/cell cycle ($n = 36$), DNA damage ($n = 9$), cell invasion/motility ($n = 19$), and other cellular signaling ($n = 31$). Tumor samples were probed and assessed, as previously described [19, 22, 23]. Lysates were extracted from each tumor specimen and four replicates from each lysate were spotted on the RPPA. Dilution curves were fitted to the logistic model “Supercurve Fitting” (<http://bioinformatics.mdanderson.org/OOMPA>). Relative protein levels for each sample were derived from the supercurve for each lysate by curve-fitting and normalized for protein loading [21], and the log₂-scaled protein concentrations were normalized by global sample median normalization [24].

Immunohistochemistry (IHC)

We cross-validated select markers identified by RPPA using well-validated IHC markers. Nuclear Cyclin D1 expression was evaluated on pre- and post-metformin-treated tissue samples, as well as matched untreated controls. Four- μ m thick sections from the tissue block were incubated for 30 min at 37 °C with anti-Cyclin D1 (SP4-R) Rabbit Monoclonal Primary antibody (Ventana, California). The antibody was diluted in 0.05-M Tris-HCl with 1 % carrier protein and ProClin 300 as a preservative. Antigen was detected with an ultraView Universal diaminobenzidine (DAB) detection kit. In addition, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) were assessed on tumor tissue, per standard ASCO-CAP guidelines [25, 26].

Western blot analysis

MDA-MB-468 (basal; hormone receptor negative and HER2 negative) and T47D (luminal A; hormone receptor positive and HER2 negative) cell lines were obtained from the American Type Culture Collection (ATCC: Manassas, VA). Cells were cultured in DMEM media and 10 % fetal bovine serum at 37 °C and 1 % penicillin/streptomycin with 5 % CO₂. Cells were passaged by 0.25 % Trypsin-EDTA when they reached approximately 80 % confluence. Metformin was obtained from Sigma-Aldrich.

Cells were lysed with 2X Laemmli buffer. 25 μ g of total proteins were separated by electrophoresis and transferred to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies followed by secondary antibodies and then developed with an enhanced chemiluminescence detection kit according to manufacturer's instructions. Antibodies obtained from the Cell Signaling Technology (Beverly, Massachusetts) included anti-phospho-AMPK^{T172} (Catalog number: 4188), anti-Raptor (Cat. no. 2280), anti-phospho-JNK^{Thr183/Tyr185} (Cat. no. 4668), and anti-phospho-Bad^{S112} (Cat. no. 5284). In addition, we assessed Cyclin D1 expression by the Santa Cruz antibody (Cat. no. 8396).

Statistical analysis

We analyzed changes in protein expression in tumor tissue of study patients with those of untreated historical controls. The RPPA spot signal intensity data from MicroVigene (VigeneTech, Inc.) were processed by the R package SuperCurve (version 1.01), as previously described [27]. Since the distribution of the original expression of the RPPA data was skewed, all statistical analyses were based on the log₂ transformation of the original data [28]. For RPPA and IHC, paired t-tests were used to calculate within-group changes, and two-sample t-tests were used to compare between-group changes in cases and controls (significance: $p < 0.05$). For RPPA, multiple comparisons were adjusted for by fixing the false discovery rate (FDR) at 25 %. All tests were two-sided at $p = 0.05$ significance level. Means and standard deviations (SDs) were generated for all tissue biomarkers. Pearson's correlation coefficient was used for correlation between RPPA and IHC. R (<http://cran.r-project.org>) was used for all analyses.

Results

Patient cohort

Of the 35 patients enrolled to the study, 33 completed the trial and were considered evaluable. The cohort was described in detail previously [10]. Briefly, of the 33 evaluable patients, 9 had DCIS and 24 had invasive carcinoma. Of the invasive tumors, 80 % of patients had a hormone receptor positive, HER2- breast tumors and 1 patient had triple negative breast cancer. The controls ($n = 34$) were well matched by age, stage, and BMI, and previously described [10]. Metformin was administered for a median of 23 days (range: 8–64). The most common side effects were grade I–II toxicities, including self-limited diarrhea, flatulence, abdominal pain, fatigue, and anorexia.

Reverse Phase Protein Array

Matched pre- and post-tissue was available from 32 metformin-treated patients and 34 untreated controls for RPPA. Unsupervised clustering of the proteomic profiles from RPPA analysis is shown in Fig. 1. Among the 160 proteins evaluated, 73 demonstrated a significant change from baseline in the metformin-treated group for log-transformed data (Supplemental Table 1), including markers from each of the 7 classification groups: angiogenesis, PI3K/AKT/AMPK signaling, apoptosis/cell cycle, DNA damage, cell invasion/motility, MAPK signaling, and other signaling. The mean change from baseline in protein expression (expressed as normalized logarithmic intensities) in the 73 differently expressed markers varied from -0.002 to $+0.365$ in the metformin group. Significant biomarker changes also occurred within the untreated control group ($n = 65$), in which the mean change from baseline ranged from -0.001 to $+0.351$.

In comparing the changes between metformin-treated cases and historical untreated controls, 18 proteins varied significantly (Supplemental Table 1). No significant differences were seen in any of the seven classification groups (PI3K/AKT/AMPK signaling, MAPK signaling, etc.); however, the number of markers within each group was small (data not shown).

After adjusting for multiple comparisons by fixing the FDR at 25 %, 11 proteins remained significantly different when comparing differences between metformin-treated cases and matched untreated controls (Table 1). A significant increase in Raptor (0.039 vs. -0.115 , $p = 0.006$) within the PI3K/AKT pathway was observed with metformin treatment compared to no treatment. For the MAPK pathway, we identified an increase in C-Raf (0.134 vs. 0.013 , $p = 0.005$), decrease in MAPK^{T202,Y204} (-1.020 vs. -0.628 , $p = 0.022$), and decrease in JNK^{T183,pT185} (-0.064 vs. 0.074 , $p = 0.007$). For the apoptosis/cell-cycle markers, there was down-regulation of Bad^{S112} (-0.049 vs. 0.172 , $p = 0.007$), increase in Cyclin B1 (0.126 vs. -0.144 , $p = 0.021$), and increase in Cyclin D1 (0.065 vs. -0.044 , $p = 0.02$). A cell invasion/motility marker, TRFC was increased (0.128 vs. -0.034 , $p = 0.008$). An increase in Syk (0.102 vs. -0.200 , $p = 0.020$), reduction in PKC.alpha^{S657} (0.090 vs. -0.093 , $p = 0.017$), and decrease in Src^{Y416} expression (-0.713 vs. -0.461 , $p = 0.001$) were also observed. When comparing only the metformin-treated ($n = 24$)-to-untreated controls ($n = 21$) in patients with invasive breast cancer, each of the 11 proteins found in the overall cohort remained significant, with the exception of PKC.alpha^{S657} (Supplemental Table 2). The

direction of the change (i.e., increase vs. decrease of protein level) remained the same in the invasive cohort (Fig. 2).

Immunohistochemistry

The primary endpoint of the overall study was reduction in tumor proliferation, as measured by ki-67, in newly diagnosed breast cancer patients treated with metformin. As discussed previously [10], there was no reduction in In(ki-67) after metformin ($p = 0.98$) or when compared to historical controls ($p = 0.47$). In addition, we assessed whether there was a reduction in tumor proliferation, as measured by PCNA (Proliferation Cell Nuclear Antigen) expression by RPPA. No changes were seen after metformin use in PCNA as compared to baseline (Table 1). There was a significant correlation between ki-67 and tumor proliferation as measured by RPPA expression of PCNA (correlation coefficient $r = 0.45$, $p = 7.96 \times 10^{-8}$) [10].

In addition, we compared protein expression of clinically significant markers between IHC and RPPA. There was a significant correlation between IHC and RPPA for ER (correlation coefficient $r = 0.77$, $p < 1 \times 10^{-16}$), PR ($r = 0.53$, $p < 1 \times 10^{-7}$), and HER2 ($r = 0.45$, $p = 0.0005$).

Given the increase identified by RPPA in Cyclin D1 levels (Table 1), we evaluated whether a similar finding was observed in IHC. There was no significant mean change in Cyclin D1 when comparing tumor tissue from metformin-treated vs. non-treated patients [-5.64 (SD: 21.54) vs. -6.37 (14.28), $p = 0.88$]. In addition, there was not a significant correlation in Cyclin D1 between IHC and RPPA ($r = 0.09$, $p = 0.31$).

Western blot analysis

To investigate whether the proteomic changes identified by RPPA were also observed in cells lines, MDA-MB-468 and T47D cell lines were treated with increasing doses of metformin (1 and 4 mM). While no change in the phosphorylation of AMPK was seen in RPPA, an increase in AMPK^{pT172} was observed in both MDA-MB-468 and T47D cell lines. AMPK phosphorylation appeared to be concentration-dependent. By RPPA, there was no significant difference in AMPK^{pT172} between metformin-treated cases vs. metformin-untreated controls (Table 1). Similar to RPPA, a dose-dependent decrease in JNK^{pT183} was seen in both cell lines. Consistent with RPPA, a reduction in Bad^{pS112} was observed, but was more apparent in MDA-MB-468 cell lines. In contrast to RPPA, no change in Raptor was identified. Finally, no change in Cyclin D1 was seen in either cell line, which was consistent with IHC but different than the increase seen in RPPA.

Discussion

In this study, we have demonstrated that treatment with metformin results in a significant change in a number of proteomic markers in overweight or obese newly diagnosed breast cancer patients. Of the 160 markers assessed by RPPA, 11 of the protein markers were significantly different before and after metformin administration, when compared to untreated patients, after adjusting for multiple comparisons. These markers reflect a wide range of oncologic activity, including PI3K/AKT signaling, apoptosis/cell cycle, and

invasion/motility. While Cyclin D1 increased by RPPA, there was no change by the IHC analysis. In addition, not all proteins that changed with RPPA were consistently modified after metformin in two breast cancer cell lines, when assessed by the western blot.

We observed an increase in TRFC and SYK by RPPA after metformin use. These associations have not been previously reported. However, a number of our RPPA findings are consistent with prior pre-clinical observations by others, performed primarily in cell lines. For instance, pMAPK reduction was identified after metformin administration in MCF-7 and BT474 cells [29]. In triple negative cell lines, metformin use associated with reduction in pMAPK and pSrc [3]. In non-small lung cancer cell lines harboring wild-type LKB1, activation of MAPK was seen after an increase in C-RAF/B-RAF heterodimerization, potentially through down-regulation of Rheb. In our study, we identified an increase in the level of C-RAF expression after metformin [30]. In regards to Src, Hirsch et al. used an inducible model of cellular transformation with mammary epithelial cells (MCF-10A) containing ER-Src, to demonstrate that metformin inhibits Src-inducible expression by blocking the earliest step in the transformation process initiated by Src [7]. In addition, we observed a reduction of activated JNK in our pre-surgical trial. Others demonstrated that metformin inhibits the migration and invasion of hepatocellular carcinoma cells through dose-dependent inhibition of JNK and ERK signaling [31]. Notably, metformin was recently identified as a potential therapeutic agent against acetaminophen-induced acute liver failure, with the proposed mechanism of action due to the sustained reduction of JNK phosphorylation in the liver [32]. In HT-1080 fibrosarcoma cells, metformin limited invasion and migration through a reduction of activated JNK as well as phosphorylated PKC alpha [33], which was demonstrated in our series as well. These pre-clinical findings are in accordance with our observations in human breast tumor tissue after approximately 2 weeks of metformin administration.

However, not all of our RPPA findings are consistent with protein changes observed by others. In pre-surgical metformin trials in breast cancer, the impact of metformin on apoptosis was variable; with one study reporting a significant increase in terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining [13] and another showing no significant change in TUNEL staining [34]. In our pre-surgical trial, the only apoptotic marker that changed was down-regulation of Bad^{pS112}. Bad, or the Bcl-2 associated death promoter, is a member of the pro-apoptotic Bcl-2 family, which also includes Bim, Bax, and Bid. Other studies demonstrated either no change in Bad levels (pancreatic cell lines, PANC-1 and BxPC3) [35] or an increase in Bad expression (ovarian epithelial cell lines, OVCAR-3, and OVCAR-4) [36] after metformin. In addition, we identified changes in cell-cycle markers that differ from the description of others. We observed an increase in the expression of the G2/M progression-associated Cyclin B1. In a human epidermoid A431 tumor xenograft model, metformin significantly reduced tumor growth, associated with a reduction of Cyclin B1 and the G1-associated Cyclin D1 [37]. Other pre-clinical studies reported a reduction in Cyclin D1 after metformin use in breast cancer (MCF-7 cells) [38], retinoblastoma (Y79 and WERI cell lines) [39], head and neck cancer (FaDU and Detroit 562 cells) [40], prostate cancer (LnCaP, DU145, and HT-29 cell lines; LNCaP xenograft model) [41], and hepatocellular carcinoma (HepG2 cell line and PLC/PRF/5 xenograft model) [42]. This finding differs from our observation that Cyclin D1

increases, per RPPA, which led us to evaluate Cyclin D1 change by the other protein expression methods.

We did not observe a significant change in Cyclin D1 after metformin use. In MDA-MB-468 and T47D, no change in Cyclin D1 by western blot was seen after metformin use. On the contrary, a reduction in JNK^{pT183} (both cell lines) and Bad^{pS112} (MDA-MB-468 cell lines) was identified, consistent with RPPA. As metformin use was previously shown to impact downstream proteins in the PI3K/AKT/mTOR pathway, we specifically tested proteins in this pathway in cell lines. For instance, metformin administration was shown to inhibit the growth of breast cancer cells through AMPK activation [5]. This finding was replicated in a number of other studies, including the recent reporting of an increase in pAMPK^{T172} in a pre-surgical metformin breast trial [43]. While no change in pAMPK^{T172} was seen by RPPA ($p = 0.821$) in our study, we identified an increase in Raptor by RPPA. In contrast, in MDA-MB-468 and T47D cell lines, we observed an increase in pAMPK^{T172} and no change in Raptor by western similar to findings by Hadad et al., using even higher doses of metformin [44]. Thus, not all of our RPPA findings are replicated using the other measures of protein expression.

There are potential advantages of RPPA over other means of assessing protein expression, such as IHC and immunoblotting. These include cost, amount of material required, objective quantification, and a superior dynamic range [21]. However, IHC provides additional description on spatial organization. In frozen tissue, others reported significant correlations in marker expression levels between RPPA and IHC, including total and phospho-AKT, ER, and PR [21]. In our study, we analyzed protein expression on FFPE. We identified a significant correlation between RPPA and IHC for ER, PR, and HER2. In addition, Meric-Bernstam et al. assessed for correlations between RPPA and IHC in 53 patients with invasive breast cancer who prospectively underwent intraoperative image-guided core-needle biopsies and central and peripheral surgical tumor specimens [22]. These samples were frozen or formalin-fixed immediately. They observed that proteomic levels were differentially impacted by biospecimen type, as well as other pre-analytic levels. Correlations between RPPA and IHC analyses were also variable. For instance, moderate-to-strong correlation was identified with pAKT, whereas the correlation of PTEN expression was weak. Beyond analytic differences, there are a number of pre-analytic variables that should be considered when performing pre-surgical trials. Discordance between marker expressions on core biopsy vs. surgical resection tissue has been reported, even with commonly used markers, such as PR levels [45]. In addition, the half-life and stability of the marker are important, particularly with phospho-proteins and with large tumor resections [46]. These variables are critical to consider when interpreting data from pre-surgical trials and interpreting proteomic findings based off of FFPE tissue should be done with caution.

Our study has several strengths. We performed a thorough investigation of proteomic marker change after metformin use, conducting RPPA followed by IHC and western blotting on select markers. In addition, we had a sufficient number of samples to compare to a control group, matching for important clinical variables. Even though the majority of our samples were small, RPPA allows protein quantification with limited tissue. Weaknesses include a relatively small sample size. In addition, the study was non-randomized, so there was no

prospective placebo group for the analyses. The majority of the tumors had a low proliferation rate. In addition, they were primarily hormone receptor positive, HER2-invasive breast cancers, and it is unclear whether these results are generalizable to other breast cancer subtypes [10]. In addition, there were a small number of patients with DCIS included in this analysis, leading to a heterogeneous population of assessed tumors. In addition, ideally, we would have collected both frozen tissue and FFPE at the time of biopsy and resection.

This study has demonstrated that pre-surgical metformin use is associated with a number of significant proteomic changes, including previously unreported changes in TRFC and Syk. Large-scale proteomic analysis should be cautiously interpreted in FFPE, with the consideration of further assessment by the other protein expression methods, such as IHC and immunoblotting. The results of this study should be further validated in a larger, prospective trial. If confirmed, associations between these changes and the impact on breast cancer outcome should be assessed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Median-centered unsupervised hierarchical cluster of breast cancer samples (pre-/post-treatment paired samples from 32 metformin-treated cases and 34 untreated matched historical controls) using quantification data for 161 proteins derived from reverse phase protein arrays (RPPA)

Fig. 2.

Changes in protein expression by the western blot analysis after treatment with metformin (up to 4 mM) There was a dose-dependent decrease in JNKpT183 and BadpS112 in both 2a) T47D and 2b) MDA-MB-468 cells. An increase in AMPKpT72 was observed. No change in raptor or cyclin D1 was identified. Actin was a control. *Met* metformin

Table 1

RPPA-generated protein expression in breast tumor tissue at baseline (pre-treatment) and following metformin treatment compared to untreated historical controls

Biomarker	Metformin-treated group		Untreated control group		p value	p value, 2-sample t test	FDR
	Baseline (SD)	Mean absolute change from baseline (SD)	Baseline (SD)	Mean absolute change from baseline (SD)			
Signaling							
PI3K/AKT/AMPK							
Raptor	−0.002 (0.248)	0.039 (0.198)	0.108 (0.290)	−0.115 (0.227)	0.008 *	0.006 **	0.18 ***
MAPK							
C.Raf	−0.079 (0.143)	0.134 (0.120)	0.012 (0.176)	0.013 (0.191)	0.697	0.004 **	0.18 ***
JNKpT183,pT185	0.047 (0.139)	−0.064 (0.203)	−0.037 (0.175)	0.074 (0.186)	0.034 *	0.007 **	0.18 ***
MAPKpT302,Y204	0.444 (0.337)	−1.020 (0.725)	0.205 (0.489)	−0.628 (0.581)	<0.001 *	0.022 **	0.24 ***
Other							
PKC.alpha ^{S657}	−0.027 (0.265)	0.090 (0.330)	0.106 (0.192)	−0.093 (0.254)	0.050 *	0.017 **	0.23 ***
Src ^{Y416}	0.558 (0.462)	−0.634 (0.545)	0.103 (0.319)	−0.350 (0.349)	<0.001 *	0.018 **	0.23 ***
Syk	0.042 (0.364)	0.102 (0.362)	0.162 (0.608)	−0.200 (0.595)	0.072	0.020 **	0.24 ***
Apoptosis/cell cycle							
Bad ^{S112}	0.079 (0.264)	−0.049 (0.334)	−0.161 (0.268)	0.172 (0.329)	0.007 *	0.011 **	0.21 ***
Cyclin_B1	0.040 (0.407)	0.126 (0.274)	0.167 (0.600)	−0.144 (0.566)	0.167	0.021 **	0.24 ***
Cyclin_D1	−0.042 (0.114)	0.065 (0.118)	0.052 (0.134)	−0.044 (0.152)	0.113	0.002 **	0.18 ***
Cell invasion/motility							
TRFC	−0.035 (0.211)	0.128 (0.243)	0.023 (0.271)	−0.034 (0.218)	0.393	0.008 **	0.18 ***

SD Standard Deviation

*

p < 0.05, paired *t* test

**

p < 0.05, 2-sample *t* test

p < 0.05, 2-sample *t* test, FDR (false detection rate) < 0.25