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The Upregulation of PI3K/Akt and MAP Kinase Pathways is Associated with Resistance of Microtubule-Targeting Drugs in Prostate Cancer

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ABSTRACT

Resistance is a significant limitation to the effectiveness of cancer therapies. The PI3K/Akt and MAP kinase pathways play important roles in a variety of normal cellular processes and tumorigenesis. This study is designed to explore the relationship of these signaling pathways with multidrug resistance in prostate cancer (PCa). The PI3K/Akt and MAP kinase pathways were investigated utilizing paclitaxel resistant DU145-TxR PCa cells and their parental non-resistant DU145 cells to determine their relationship with resistance to paclitaxel and other anticancer drugs. Our results demonstrate that the PI3K/Akt and MAP kinase pathways are upregulated in DU145-TxR cells compared to the DU145 cells. Inactivating these pathways using the PI3K/Akt pathway inhibitor LY294002 or the MAP kinase pathway inhibitor PD98059 renders the DU145-TxR cells more sensitive to paclitaxel. We investigated the effects of these inhibitors on other anticancer drugs including docetaxel, vinblastine, doxorubicin, 10-Hydroxycamptothecin (10-HCPT) and cisplatin and find that both inhibitors induces DU145-TxR cells to be more sensitive only to the microtubule-targeting drugs (paclitaxel, docetaxel and vinblastine). Furthermore, the treatment with these inhibitors induces cleaved-PARP production in DU145-TxR cells, suggesting that apoptosis induction might be one of the mechanisms for the reversal of drug resistance. In conclusion, the PI3K/Akt and MAP kinase pathways are associated with resistance to multiple chemotherapeutic drugs. Inactivating these pathways renders these PCa cells more sensitive to microtubule-targeting drugs such as paclitaxel, docetaxel and vinblastine. Combination therapies with novel inhibitors of these two signaling pathways potentially represents a more effective treatment for drug resistant PCa. J. Cell. Biochem. 116: 1341–1349, 2015.

KEY WORDS: PROSTATE CANCER; MULTIDRUG RESISTANCE; PI3K/Akt; MAPK; PARP

Chemotherapeutics are widely used for the treatment of advanced human cancers. While chemotherapies are often initially effective, long-term treatment leads to drug resistance [Holohan et al., 2013]. Drug resistance is a crucial problem in cancer therapy which impacts mortality rates as well as the healthcare costs of patients worldwide. However, the mechanisms underlying drug resistance are not fully understood [Baguley, 2010].

Prostate cancer (PCa) is the most common cancer in male in the United States, and also the second leading cause of cancer-related deaths in men [Siegel et al., 2014]. While advanced PCa is often initially responsive to the withdrawal of androgens, men with the disease often progress to castration resistant PCa (CRPC), an androgen-independent state [Trewartha and Carter, 2013]. Studies have demonstrated that chemotherapeutic drugs such as paclitaxel and docetaxel, provide a modest survival advantage when used in men with CRPC, but most of these men develop resistance to chemotherapy and progress. There are currently no effective approaches for treating drug resistant PCa [Seruga et al., 2011; Hwang, 2012].

Several important molecules and signaling pathways have been shown to contribute to the resistance of cancer cells to chemotherapeutic agents [Turner and Reis-Filho, 2012]. The JNK1/c-jun signaling pathway was reported to be involved in ABCG2-mediated multidrug resistance in colon cancer cells. Blocking the JNK pathway

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with the pathway inhibitor SP600125 reduces the expression level and transport function of ABCG2 in drug-resistant SW116/HCPT cells [Zhu et al., 2012]. H69A lung cancer cells with multidrug resistance have been demonstrated to have significantly higher Nrf2-ARE pathway activity and expression of the *Mrp1* gene. Inhibiting the activity of Nrf2 level restored the sensitivity to drug treatments [Ji et al., 2013]. Notably, the PI3K/Akt pathway plays a fundamental role in the regulation of cell growth, division, survival, and, when disarranged, tumorigenesis. Signaling of this pathway activates the Ser/Thr protein kinase Akt through phosphorylation, which in turn phosphorylates and regulates downstream effector proteins and promotes cell proliferation and survival [Vivanco and Sawyers, 2002; Hennessy et al., 2005]. The MAP kinase pathway, similarly, plays a fundamental role in the regulation of cell proliferation, differentiation, and survival. Aberrant activation of this pathway through, for example, a BRAF mutation is associated with tumorigenesis [Dhillon et al., 2007; Roberts and Der, 2007]. Decreased activation of the MAP kinase pathway is associated with significant suppression of breast cancer cell growth in vitro and tumorigenesis in vivo [Cipriano et al., 2014]. Additional data also suggest that the PI3K/Akt and MAP kinase pathways play important roles in drug resistance in human cancers [West et al., 2002; McCubrey et al., 2007; Pritchard and Hayward, 2013].

Human tumors are highly heterogeneous involving multiple types of epithelial, endothelial and stromal components and, as such, the mechanism of drug resistance can vary in distinct types of cancers. PCa is an example of a malignancy with such heterogeneity. By investigating PI3K/Akt and MAP kinase pathways on paclitaxel-resistant PCa cells and its parental non-resistant cells, we attempted to identify whether these two signaling pathways are associated with mechanisms of drug resistance.

MATERIALS AND METHODS

CELL LINES AND CULTURE CONDITIONS

The paclitaxel resistant PCa cells DU145-TxR and its parental non-resistant DU145 cells were provided as a gift by Dr. Namiki and Dr. Mizokami (Department of Urology, Kanazawa University, Kanazawa Japan) [Takeda et al., 2007]. The DU145 and DU145-TxR cells were maintained in Eagle′s Minimum Essential Medium (ATCC, Manassas, VA) supplemented with 10% FBS (Life Technologies, Grand Island, NY). The DU145-TxR cells were cultured in 10 nM paclitaxel (Cayman Chemical, Ann Arbor, MI) in order to maintain their drug-resistant phenotype. Prior to each experiment, these cells were grown for a minimum of 1 week in paclitaxel free medium.

Under these culture conditions, cells were treated, where indicated, with the Akt inhibitor LY294002 (Enzo Life Sciences, Farmingdale, NY) or the MEK inhibitor PD98059 (Enzo Life Sciences) at several concentrations. DMSO and ethanol were used in parallel as the vehicle control.

MUTATION ANALYSIS

Genomic DNA sequencing of specific genes in DU145 and DU145-TxR cells was performed to compare the mutational status. For *H-Ras*, *K-Ras*, *N-Ras*, *PIK3Ca* and *BRAF* genes, genomic DNA was amplified using the same primers and PCR conditions as previously described [Liu et al., 2008]. After PCR amplification of the specific exons, Big-dye reaction was performed for DNA sequencing. The primers used in PCR amplification and Big-dye sequencing for these genes are summarized in Supplementary Table S1.

REAL-TIME PCR

Total RNA from the DU145 and DU145-TxR cells was isolated using the TRIzol reagent (Life Technologies) following the manufacturer′s instructions. Three micrograms of total RNA was reverse-transcribed using Oligo-dT primers and superscript III RT (Life Technologies). The expression of the genes in the MAP kinase and PI3K/Akt pathways was identified using the signaling pathway PCR Array (Qiagen, Valencia, CA) on a CFX96 Touch Real-Time PCR Detection System (Bio-rad, Hercules, CA) according to the manufacturer′s instructions. The genes demonstrating alterations in expression based upon the PCR Plate Array were confirmed using quantitative real-time PCR. The primers were purchased directly from Qiagen (Valencia, CA).

WESTERN BLOT ASSAY

Protein extracts were isolated from DU145 and DU145-TxR cells in RIPA buffer (Thermo Fisher Scientific, Waltham, MA) with standard protease inhibitors (Thermo Fisher Scientific, Rockford, IL). Western blot analyses were performed as described previously [Liu et al., 2008] using primary antibodies, including anti-phospho-Akt (Cat. No. 9271s), anti-phospho-ERK (Cat. No. 4370s), anti-total-Akt (Cat. No. 9272s), anti-total-ERK (Cat. No. 9194s), anti-PARP (Cat. No. 9542s), anti-cleaved-PARP (Cat. No. 9541s) from Cell Signaling (Beverly, MA), anti-MDR1 (Cat. No. MAB4120, EMD Millipore, Billerica, MA) and anti-β-actin (Cat. No. sc-130065, Santa Cruz, Dallas, TX). Each experiment was replicated at least three times with similar results and only the representative figures of western blot assay were shown.

CELL VIABILITY ASSAY

DU145 or DU145-TxR cells exposed to different treatments were seeded in 96-well plates and cultured with indicated concentration of chemotherapeutic drugs: paclitaxel, docetaxel, vinblastine, doxorubicin, 10-HCPT or cisplatin (Cayman, Ann Arbor, MI). After 72 h, the cell proliferation reagent WST-1 (Roche Diagnostics, Indianapolis, IN) was added to the wells and incubated for another 2 h. Cell viability and the IC_{50} for the specific drug were measured and calculated subsequently. For each condition of treatment, at least three replicates were done and the average of these data were used for data analysis.

STATISTICAL ANALYSIS

For the re-sensitization of DU145-TxR cells to paclitaxel by LY294002 or PD98059 treatment, one way ANOVA and Holm-Sidak′s multiple comparisons with control group was applied using GraphPad Prism version 6.07 for Windows (GraphPad Software, La Jolla, CA). For the drug resistance analysis of paclitaxel, doxorubicin, docetaxel, 10-HCPT, vinblastine and cisplatin after treatment of LY294002 or PD98059, nonparametric unpaired *t* test (Mann–Whitney test) was performed using the same software. The cell viability assay for resistance of DU145 and DU145-TxR cells to paclitaxel were analyzed using unpaired *t* test at each concentration. The statistical significance level was set at $P < 0.05$ for all analysis.

RESULTS

UPREGULATION OF THE PI3K/AKT AND MAP KINASE PATHWAYS IN PACLITAXEL RESISTANT DU145-TxR CELLS

The paclitaxel resistant PCa cell line, DU145-TxR, was established from DU145 cells using a stepwise treatment with paclitaxel for 9 months. The resulting cells were more resistant than the parental cells and were named DU145-TxR [Takeda et al., 2007]. Although previously established, the IC_{50} of paclitaxel in the DU145 and DU145-TxR cells was evaluated. As shown in Supplementary Figure S1, DU145-TxR cells were much more resistant to paclitaxel than DU145 cells, with the IC_{50} of 608 and 8 nM, respectively. There are more DU145-TxR cells survive after paclitaxel treatment starting from as low as 1 nM ($P < 0.05$). Moreover, the resistance of DU145-TxR cells were checked by western blot assay of MDR-1 (Fig. 1), a protein usually upregulated in drug resistant cells and was used as a positive marker for drug resistance [Brambila-Tapia, 2013].

To explore the relevance of the PI3K/Akt and MAP kinase pathways to paclitaxel resistance in PCa, total protein was isolated from the DU145 and DU145-TxR cells and western blot assay was performed to examine the expression of pathway components. DU145-TxR cells were maintained in medium with 10 nM paclitaxel to retain their resistant phenotype. In order to diminish the influence of simultaneous paclitaxel treatment on the findings presented here, the DU145-TxR cells were cultured in normal medium without paclitaxel for 1 week prior to their being studied. The phosphorylation of Akt (p-Akt) and ERK (p-ERK) was compared between the paclitaxel resistant DU145-TxR cells and non-resistant DU145 cells. As shown in Figure 2, both p-Akt and p-ERK levels were significantly upregulated in DU145-TxR cells, but total Akt and ERK levels were similar in both cell lines. Since p-Akt and p-ERK levels represent the activity of the PI3K/Akt and MAP kinase pathways respectively, the results suggest that both signaling pathways were upregulated in drug resistant cells.

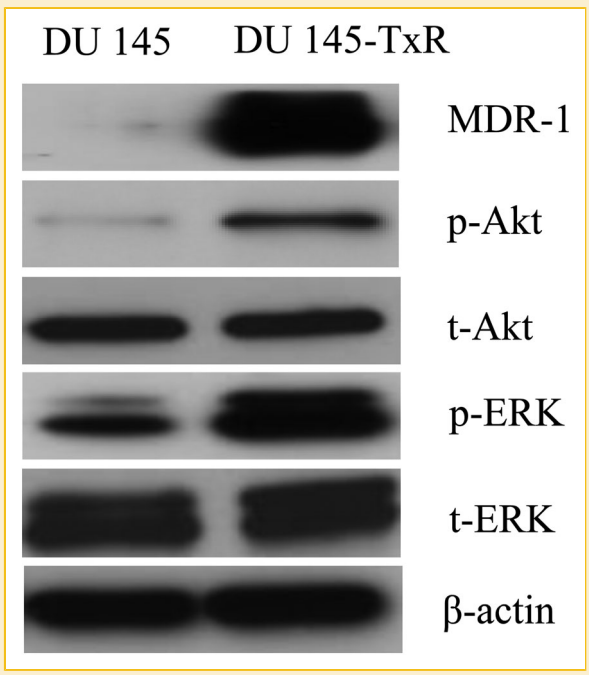

Fig. 1. PI3K/Akt and MAP Kinase pathways were significantly upregulated in DU145-TxR cells. DU145 and DU145-TxR cells were cultured in medium without paclitaxel for 1 week and total protein isolated. P-Akt and p-ERK, which were used to represent the activation of these two signaling pathways, were detected by Western blot along with t-Akt and t-ERK, and MDR-1. β-actin was used as an internal control. DU145-TxR: treatment resistant DU145 cells.

GENETIC ALTERATION IN DU145-TxR Cells

Since both the PI3K/Akt and MAP kinase pathways were upregulated in the paclitaxel resistant DU145-TxR cells, we explored several important genetic alterations of these two pathways and compared their expression in order to identify the mechanism of the activation of these pathways. *H-Ras, K-Ras, N-Ras, PIK3Ca* and *BRAF* genes were selected for mutational analysis but no differences between the DU145-TxR and DU145 cells were observed. Subsequently, PCR arrays and real-time PCR specific to these signaling pathways were utilized in order to detect the expression of several key regulatory genes of these two pathways. These included *EGFR*, *VEGFR2*, *c-MET*, *N-Ras*, *MEK1*, *ERK1*, *PIK3Ca*, *PDK1*, *PTEN*, *AKT1*, *AKT2*, *AKT3*, etc. Only *PTEN* was identified to be significantly downregulated in DU145-TxR cells (cut-off: ≥threefold change) compared to the DU145 cells, a result which was confirmed using western blot assay (Supplementary Fig. S2). Since PTEN negatively regulates the PI3K/Akt pathway by terminating p-Akt signaling [Wu et al., 1998], the downregulation of PTEN might be one of the reasons to explain the upregulation of PI3K/Akt pathway in DU145-TxR cells.

INHIBITION OF THE PI3K/AKT OR MAP KINASE PATHWAYS INDUCES DU145-TxR CELLS SENSITIVE to PACLITAXEL TREATMENT

In order to study the relationship of these two signaling pathways with paclitaxel resistance in the DU145-TxR cells, LY294002 and PD98059, the specific inhibitors of PI3K/Akt pathway and MAP kinase pathway respectively, were utilized to downregulate the activity of these two pathways in the DU145-TxR cells and examine their relationship with drug resistance. As shown in Figure 3a–b, both LY294002 and PD98059 significantly inhibited the expression of p-Akt and p-ERK, respectively, in DU145-TxR cells. When treated with these two inhibitors, the IC_{50} of paclitaxel was decreased in a dose-dependent manner in DU145-TxR cells. As shown in Figure 3c–d, 30 μM LY294002 or PD98059 treatment can decrease the IC_{50} of resistant cells to only 1/7 (123/885 nM) or 1/4 (202/885 nM) of the original IC_{50}, respectively. Our results suggest that the inhibition of PI3K/Akt or MAP kinase pathways induces the resistant cells to become more sensitive to paclitaxel treatment.

INHIBITION OF THE PI3K/AKT OR MAP KINASE PATHWAYS INDUCES DRUG RESISTANT CELLS SENSITIVE TO MULTIPLE DRUGS

DU145-TxR cells were previously established by continuous culturing in paclitaxel for a long period of time. The cells demonstrate resistance not only to paclitaxel, but also to some other anticancer drugs, including docetaxel, vinblastine and doxorubicin [Takeda et al., 2007]. Since the inhibitors of PI3K/Akt and MAP kinase

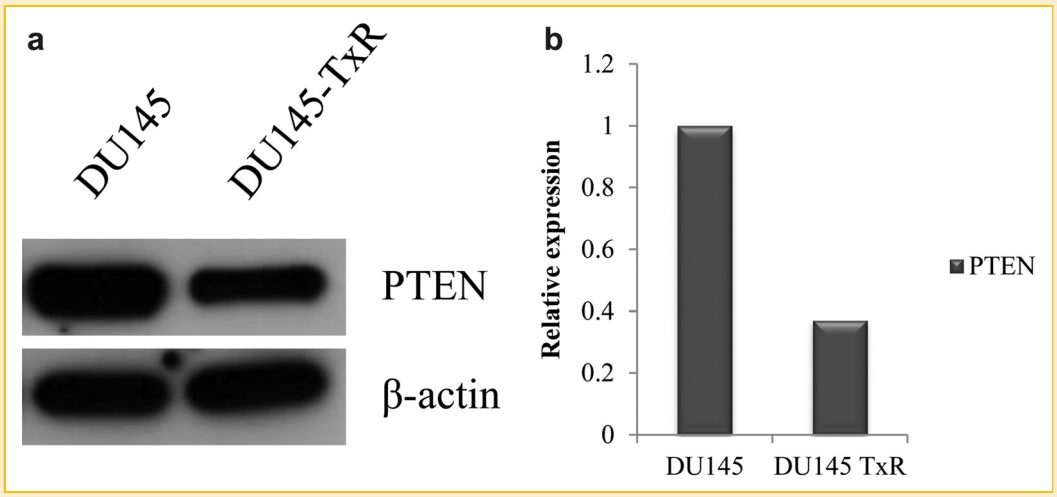

Fig. 2. Decreased expression of PTEN in DU145-TxR cells. Western blot (a) results showed that PTEN was downregulated in DU145-TxR cells resistant to paclitaxel. The quantification of Western blot band density was shown in (b). DU145-TxR: treatment resistant DU145 cells.

pathways induce the resistant cells to become more sensitive to paclitaxel (Figs. 3c–d and 4a, $P < 0.05$), we determined whether these inhibitors had similar effects on other drugs. The IC_{50} of inhibitors, LY294002 and PD98059, on the DU145-TxR cells were determined to be 15 and 50 μM (Supplementary Fig. S2) and there is no significant difference in cell viability between DU145 and DU145-TxR cells after the treatment of both inhibitors. To avoid any significant direct cytotoxic effects of these pathway inhibitors, 5 μM of each was

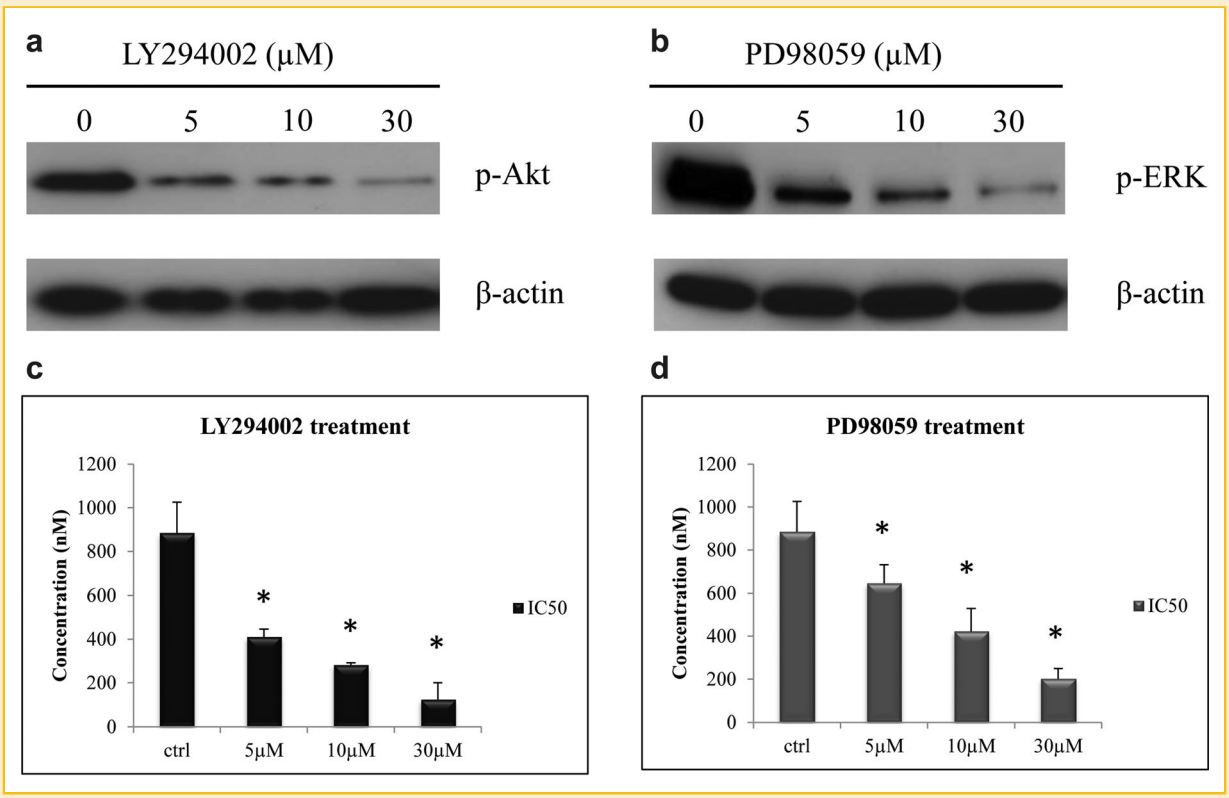

Fig. 3. Re-sensitizing DU145-TxR cells to paclitaxel using inhibitors of the PI3K/Akt and MAP kinase pathways. The DU145-TxR cells were treated with varying concentration of LY294002 and PD98059, the inhibitors of PI3K/Akt and MAP Kinase pathways respectively, for 24 h. The expression of p-Akt and p-ERK was detected by Western blot after treatment of both inhibitors (a,b). The IC_{50} of paclitaxel in DU145-TxR cells was calculated at same time with the treatment of LY294002 and PD98059 (c,d). Data was shown as mean ± SD. *Means $P < 0.05$ according to one way ANOVA and Holm-Sidak's multiple comparisons with control group. IC50: half maximal inhibitory concentration.

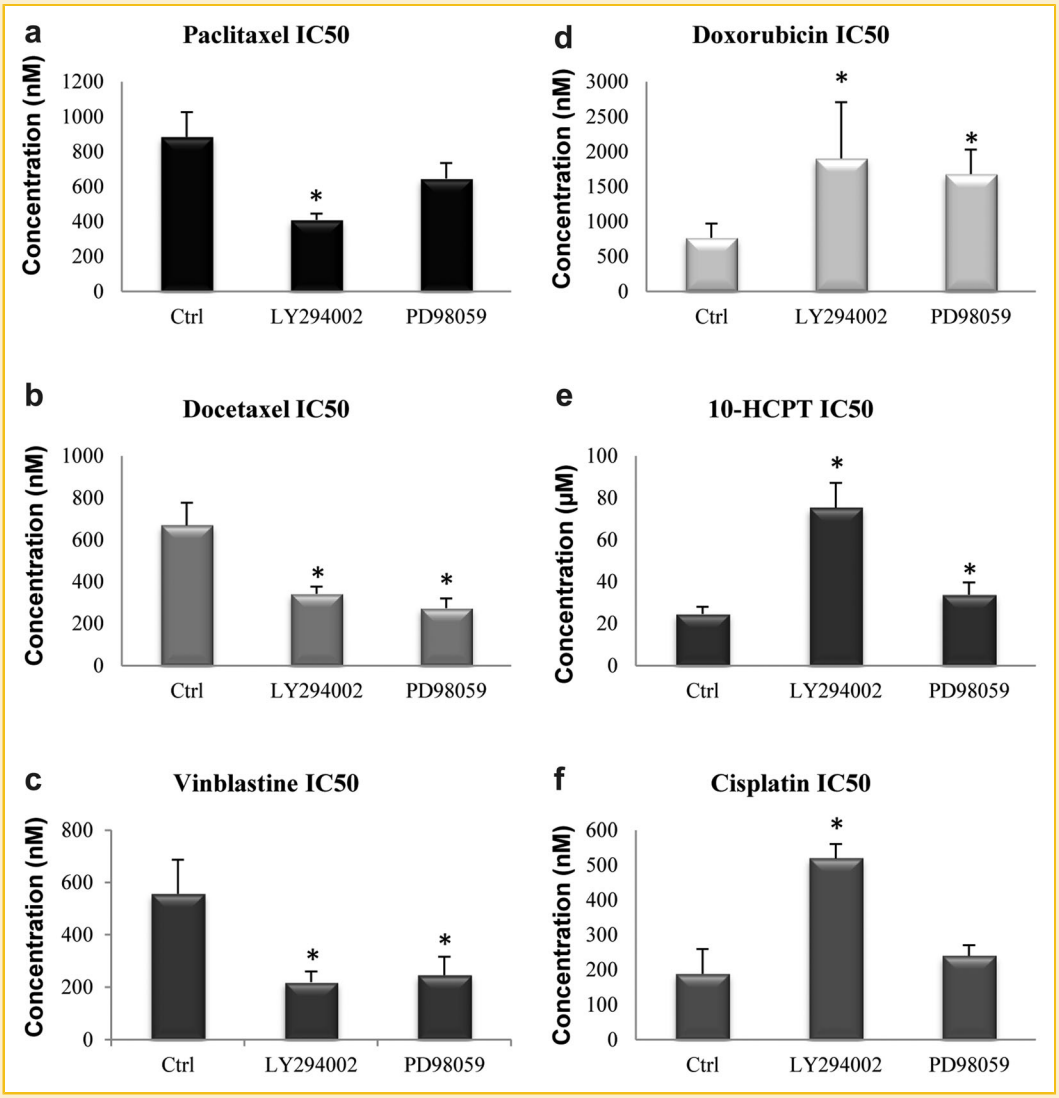

Fig. 4. Re-sensitization of DU145-TxR cells to multiple anticancer drugs targeting microtubules. The DU145-TxR cells were treated with 5 μM LY294002 or PD98059 for 24 h to inhibit the activity of PI3K/Akt and MAP Kinase pathways respectively. The IC_{50} of paclitaxel, docetaxel, vinblastine, doxorubicin, 10-HCPT and cisplatin was calculated before and after the treatment to compare the effects of the treatment to the IC_{50}. Data was shown as mean ± SD. *Means $P < 0.05$ according to *t* test compared with control group. 10-HCPT: 10-Hydroxycamptothecin; IC50: half maximal inhibitory concentration.

utilized in these studies. The IC_{50} of docetaxel, vinblastine, doxorubicin, 10-HCPT and cisplatin with or without these inhibitors was determined. As shown in Figure 4, in addition to paclitaxel, LY294002 or PD98059 induces DU145-TxR cells to be more sensitive to docetaxel, with the IC_{50} decreasing from 670 to 343 nM for LY294002 treatment and from 670 to 275 nM for PD98059 treatment (Fig. 4b, $P < 0.05$). The IC_{50} of vinblastine showed similar results and the IC_{50} decreased from 557 to 220 nM for LY294002 treatment and from 557 to 246 nM for PD98059 treatment (Fig. 4c, $P < 0.05$). However, we did not find this effect for doxorubicin, 10-HCPT and cisplatin. The IC_{50} of doxorubicin, 10-HCPT or cisplatin even increased after treatment with the inhibitors (Fig. 4d–f, $P < 0.05$). These findings suggest that the inhibitors of the PI3K/Akt or MAP kinase pathways can make PCa resistant cells more sensitive to multiple anticancer drugs, but that effect is not universal.

CLEAVED-PARP MAY BE ASSOCIATED WITH THE REVERSAL OF DRUG RESISTANCE IN DU145-TxR CELLS BY INHIBITION OF PI3K/Akt OR MAP KINASE PATHWAYS

In order to explore the mechanisms associated with the observed apparent reversal of drug resistance, we examined several important proteins and found that the expression of PARP was downregulated and cleaved-PARP was simultaneously upregulated in DU145-TxR cells after LY294002 or PD98059 treatment (Fig. 5a). However, when we detected PARP and cleaved-PARP expression in DU145 cells after LY294002 or PD98059 treatment, we did not find their expression was significantly changed at the same concentrations, which might correlate with their insensitivity to the inhibitors considering the stable p-Akt or p-ERK levels after treatment. We further increased the concentration of both inhibitors to 30 and 50 μM and found that both pathways were successfully inhibited but that the expression of PARP

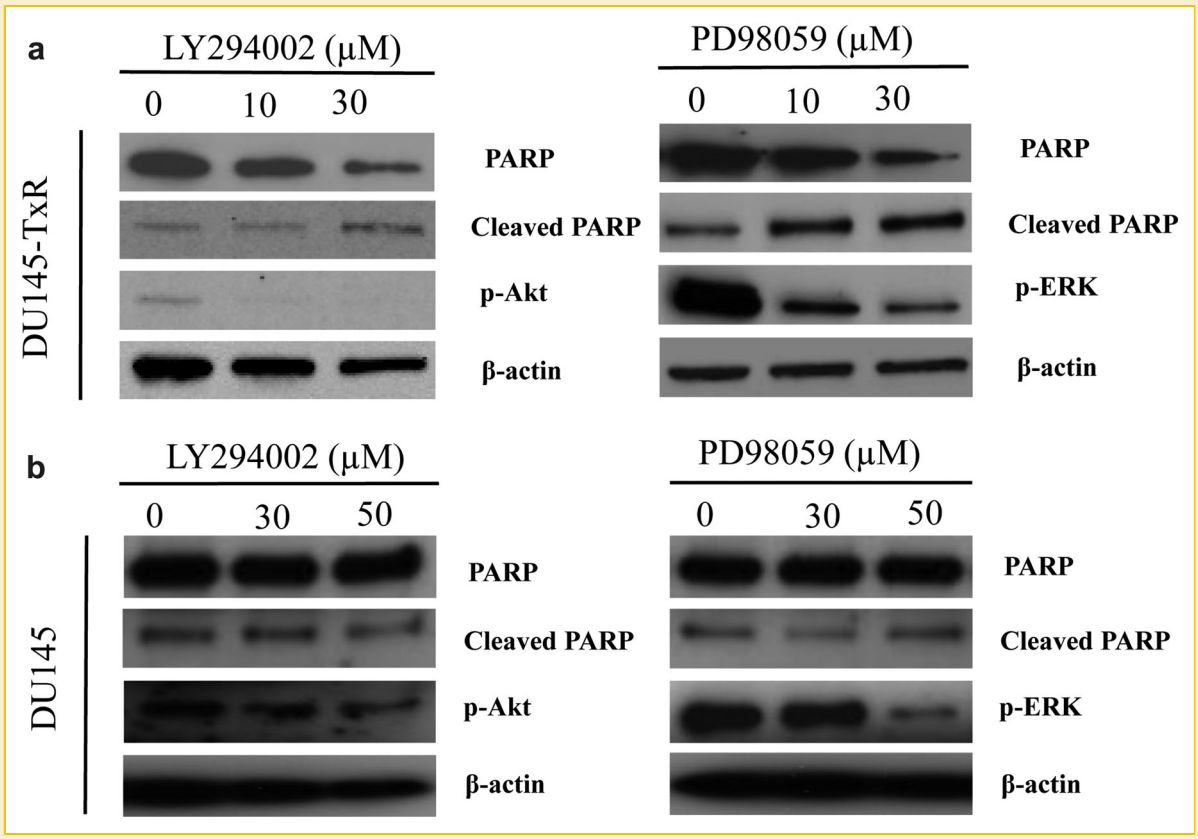

Fig. 5. Cleaved PARP is associated with the reversal of drug resistance in DU145 TxR cells. Both DU145 and DU145-TxR cells were treated with LY294002 or PD98059 for 24 h, respectively. The expression of PARP, cleaved-PARP, p-Akt or p-ERK and β-actin were detected by Western blot assay. DU145-TxR: treatment resistant DU145 cells.

and cleaved-PARP was not significantly altered (Fig. 5b). Since cleaved-PARP is an important marker for apoptosis, and the upregulation was only shown after LY294002 or PD98059 treatment in DU145 TxR cells, these findings suggest that cleaved-PARP associated apoptosis might be one of the mechanisms through which the inhibitors of PI3K/Akt and MAP kinase pathways make the resistant cells sensitive to drug treatment in PCa.

DISCUSSION

Chemotherapeutic resistance is a major obstacle to the successful treatment of cancer and at least two mechanisms of acquired resistance to taxanes have been previously characterized: (1) some tumors contain α- and β-tubulin with an impaired ability to polymerize into microtubules and have an inherently slow rate of microtubule assembly that is normalized by the Taxol [Cabral et al., 1983]; and (2) amplification of membrane phosphoglycoproteins functions as drug-efflux pumps [Gupta, 1983; Horwitz et al., 1993; Zhu et al., 2013]. Very recently, in studies of multi-drug resistance in PCa cells (22RV1), Liu et al. [2010] determined that a possible mechanism of aggressive tumor growth and drug resistance may involve the remarkably hypomethylated CpG islands on the *ABCG2* promoter of CD117+/ABCG2+ cells and found they were hypomethylated.

The paclitaxel-resistant PCa cell line DU145-TxR was previously established from DU145 cells by a stepwise treatment with paclitaxel for 9 months [Takeda et al., 2007]. We confirmed the resistance of our DU145-TxR cells by cell viability assay after paclitaxel treatment and western blot assay of MDR-1 (Supplementary Fig. S1 and Fig. 1). We screened common genetic alterations associated with paclitaxel-resistant DU145-TxR cells and found that PTEN expression was decreased by more than half in DU145-TxR cells compared with DU145 cells (Fig. 2), which suggests that it may represent a reasonable model for advanced PCa considering PTEN loss is observed in more than 40% of PCas [Steck et al., 1997; Li et al., 2013]. Since PTEN negatively regulates the PI3K/Akt pathway by terminating p-Akt signaling, the downregulation of PTEN might be one of the reasons to explain the activation of PI3K/Akt pathway, whether PTEN inhibits MAPK pathways is not known, although there was report shown that MAPK pathway activation in PTEN loss background promote PCa advancement [Mulholland et al., 2012].

When treated with LY294002 and PD98059, inhibitors of the PI3K/Akt and MAP kinase pathways at the lower non-toxic concentrations, respectively, the IC_{50} of paclitaxel was significantly decreased in a dose-dependent manner in DU145-TxR cells (Fig. 3), suggesting that upregulation of both signaling pathways were involved in paclitaxel resistance. We extended this research by screening five more chemotherapeutic agents. Interestingly, docetaxel and vinblastine

showed similar reversal of resistance when treated with the two-kinase inhibitors (Fig. 4). The synergistic effect of Akt inhibition and docetaxel are consistent with previous in vitro data of C4-2AT6 cells, which is a castration resistant PCa cell line and in vivo data of mouse xenograft [Yasumizu et al., 2014]. A similar effect was shown in gastric cancer and ovarian cancer as well [Yang et al., 2012; Li et al., 2013]. However, we did not find such synergistic effect for doxorubicin, 10-HCPT and cisplatin (Fig. 4d–f). When it comes to the mechanisms of these six anticancer drugs, paclitaxel and docetaxel exert their anticancer function through binding to microtubules and prevent their disassembly during mitosis [Yvon et al., 1999]. Vinblastine is another microtubule targeting anticancer drug. At very low concentrations, vinblastine suppress microtubule dynamics while at higher concentrations they reduce microtubule polymer mass. It also induces microtubule fragments by stimulating microtubule minus-end detach from the organizing centers [Jordan and Wilson, 2004]. On the contrary, cisplatin crosslinks DNA; doxorubicin interacts with DNA and inhibits macromolecular biosynthesis [Tacar et al., 2013] while 10-HCPT inhibits the topoisomerase I [Ping et al., 2006]. Since all three drugs that showed increased sensitivity after inhibition of the two pathways share the same anticancer mechanism by targeting the microtubules, our data suggests that the inhibitors of PI3K/Akt and MAP kinase pathways at non-toxic concentrations induces PCa resistant cells to become sensitive to multiple anticancer drugs by targeting microtubules. The effect of such specific inhibitors for drug resistance is not universal. It is worth noting that the synergistic effect of Akt inhibition with docetaxel, but not cisplatin occurs not only in PCa cell lines as shown here, but also in lung cancer cell lines as reported previously [Riedel et al., 2008], suggesting it may be a common phenomenon among cancers of various origins. Furthermore, overexpression of constitutively activated Akt decreases paclitaxel-induced cell growth inhibition in ovarian cancer, providing another direct proof of Akt pathway in the regulation of paclitaxel resistance [Yang et al., 2012]. Therefore, inhibition of PI3K/Akt and MAP kinase pathways may offer a new therapeutic method to overcome paclitaxel, docetaxel, and vinblastine resistance and permit continued treatment using this approach.

We further explored the mechanism associated with the re-sensitization of these microtubule-targeting drugs. PARP is a family of enzymes for poly (ADP-ribose) polymerase. PARP1 is the primary member of this family ubiquitously expressed in nucleus and has its prognostic value for multiple human cancers. Apart from mediating DNA damage response pathways, PARP is critical in the regulation of genomic stability, regulation of oncogenes and tumor suppressor genes expression through transcription and therefore associates with tumorigenesis, metastasis, etc. [Schiewer and Knudsen, 2014]. Preliminary investigations suggest that cleaved-PARP, a marker for apoptosis, may be involved in the action of the two-kinase inhibitors (LY294002 or PD98059) [Kunnimalaiyaan et al., 2006]. We found that cleaved PARP levels elevated significantly after exposure to inhibitors of both PI3K/Akt and MAP kinase pathways and this may in part help to explain the partial reversal of drug resistance. Clearly, other potential molecular mechanisms (such as upregulation of MDR-1 [Zhu et al., 2013]) as well as expanded analysis of the reversal of drug resistance in additional cell lines both in vitro and in vivo would be interesting to study and eventually translate our results into clinical practice.

Based upon our findings, we speculate a model of multi-drug resistance mechanism in PCa (Fig. 6). PTEN loss (and also maybe some other tumor suppressors like p53) provides the first hit while PI3K/Akt, and MAP kinase pathway activation promotes the progression of PCa drug resistance. This also occurs in other cancer types, like gastric cancer, considering the PTEN loss and PI3K/Akt activation in the PDGCX model is resistant to docetaxel as well [Li et al., 2013]. Inhibition of either pathway likely reverses the resistance of chemotherapy probably through the cleavage of PARP and other mechanisms. The detailed mechanism of PARP cleavage in the

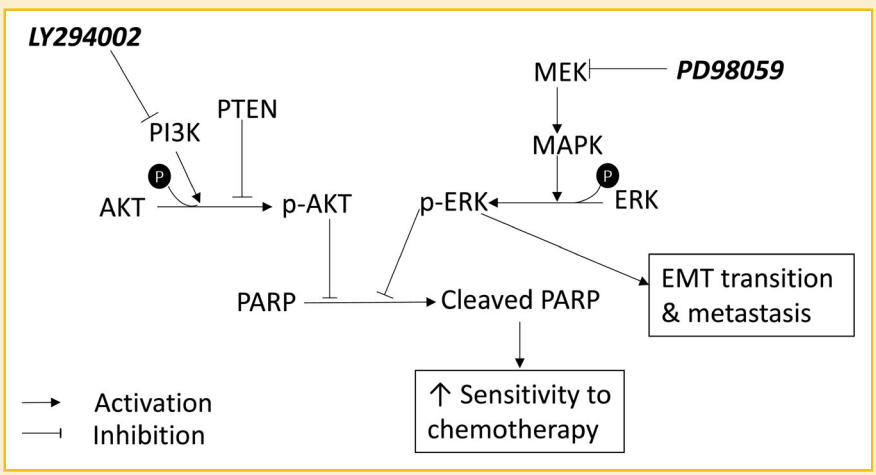

Fig. 6. Regulation of chemotherapeutic sensitivity by PI3K/Akt and MAP Kinase pathway inhibitors. PI3K/Akt and MAPK pathway activation is associated with PCa progression and resistance to chemotherapy. Inhibition of both pathways contributes to the re-sensitization of chemotherapy drugs (paclitaxel, docetaxel, vinblastine), which is associated with PARP cleavage.

increase in the sensitivity to chemotherapy is unknown. We speculate that this might be through apoptosis of the cells. It was reported that PARP inhibition by olaparib slows down cell growth in MEFs with PTEN loss at either one or two alleles [Gonzalez-Billalabeitia et al., 2014]. Similarly, our DU145-TxR cell line possesses significantly decreased PTEN expression. We think PI3K/Akt pathway inactivation along with PTEN function together could be a synergistic way to treat advanced PCa (Fig. 6), which is also supported by the synergistic functions of PTEN and PI3K/Akt pathways in the progression of PCa reported in a previous study [Gonzalez-Billalabeitia et al., 2014]. Since PTEN negatively regulates the PI3K/Akt pathway by terminating the p-Akt signaling, the downregulation of PTEN might be one of the reasons to explain the activation of PI3K/Akt pathway, whether PTEN inhibits MAP kinase pathways is not known. Notably, the recovery of drug responsiveness occurs only in microtubule targeting drugs, while resistance to other drugs is observed. Considering the complex molecular heterogeneity of PCa, we are not surprised with this finding. It was reported that inhibition of PARP or PI3K alone could stabilize tumor in a short period of time (30–40 day), while combination of both inhibitors extend the stabilization period significantly and induced robust tumor regression [Gonzalez-Billalabeitia et al., 2014]. Therefore, our data show that current or new treatments that promote inhibition of PI3K/Akt pathways should facilitate chemotherapeutics targeting microtubules. We speculate that current or new drugs that target PARP may further enhance the efficacy of drugs targeting microtubules.

There might also be some other genetic or epigenetic alterations, which activated these two signaling pathways specifically in DU145-TxR cells but were not identified in our study. Clearly, more work will be required to validate this conclusion as well as studies of other possible mechanisms related to epigenetic and other pathways to reverse paclitaxel resistance [O′Neill et al., 2011; Seruga et al., 2011]. Additionally, finding new ways to reverse drug resistance for other key chemotherapeutic anticancer drugs by identifying mechanisms that may impede drug resistance should continue to be an imperative for approved anticancer drugs.

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