Linking the immunophilin FKBP5 to taxol resistance in ovarian cancer

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Received: March 04, 2015
Published online: April 22, 2015

Taxol is a chemotherapeutic drug used to treat a number of cancers, including ovarian and breast cancers. However, taxol resistance limits treatment efficacy in cancer patients. To study the molecular mechanism of chemoresistance in ovarian cancer, we established taxol-resistant cells derived from the SKOV3 ovarian carcinoma cell lineage. Transcriptomic analysis identified 112 highly up-regulated genes in taxol-resistant cells. Among them, FK506-binding protein 5 (FKBP5) was transiently up-regulated 100 fold in taxol-resistant cells but showed reduced expression following prolonged culture. FKBP5 silencing sensitized taxol-resistant cells to taxol, while overexpression of FKBP5 increased resistance to the drug. We observed that several of the newly identified taxol resistance genes were transcriptionally regulated by FKBP5, and silencing of these genes sensitized cells to taxol. Our experiments also revealed that FKBP5 forms a protein complex with the androgen receptor (AR), and this complex regulates the transcriptional activity of both proteins. In addition, we observed that the Akt kinase pathway is regulated by FKBP5. These results indicate that the FKBP5/AR complex may affect cancer cell sensitivity to taxol by regulating expression of taxol resistance genes. Our results suggest that taxol should not be used against ovarian cancer when the Akt/FKBP5/AR axis is activated.

Keywords: Akt; androgen receptor; immunophilin; FKBP5; taxol resistance


Ovarian cancer is a common gynecological cancer in developed countries. The introduction of platinum compounds was an important step for the treatment of this malignancy. The combination of taxol and common chemotherapy was shown to improve the treatment of advanced epithelial ovarian cancer1. Nonetheless, a large fraction of patients show minimal improvements following this treatment, and the patients who benefit from this therapy may eventually relapse. The molecular mechanism underlying chemotherapeutic drug resistance is not fully understood, especially for taxol resistance. In a recent study, we used taxol-resistant ovarian cancer cell lines derived from the SKOV3 cell lineage to identify and characterize novel targets and kinase signaling pathways involved in taxol resistance.

Taxol and ovarian cancer

Taxol is a chemotherapeutic drug used to treat a number of cancers, including ovarian and breast cancers. Taxol and docetaxel are part of the taxane family of drugs. Taxol stabilizes microtubules and prevent their disassembly, therefore producing abnormalities in mitotic spindle formation, chromosome segregation, and cellular division. Drug resistance has been a major obstacle in cancer therapy, and numerous clinical studies have revealed that multidrug
FKBP5 hyperactivity in taxol resistance

FK506 binding protein 5 (FKBP5) represents a protein of the immunophilin family and is also a peptidyl-prolyl cis/trans isomerase (PPIases) involved in rotating peptide bonds directly from the cis conformation to trans. For this reason, the co-chaperone FKBP5 plays an important role in protein folding and transportation. One of the major functions of FKBP5 is to modulate steroid receptor activity (e.g., receptors for progesterone, androgens and glucocorticoids) by interacting with heat shock proteins (e.g., Hsp90/Hsp70) [24,25].

Recent studies on the role of FKBP5 in regulating apoptosis showed that FKBP5 silencing overcomes resistance to apoptosis in acute lymphoblastic leukemia [26], melanoma [28], and glioma [30]. Furthermore, FKBP5 promotes activation of genes responsible for epithelial-to-mesenchymal transition (EMT), and it also improves migration and invasion of melanoma cells [29]. In addition to protein trafficking, FKBP5 is also involved in regulating Akt activity. FKBP5 can facilitate PHLP-mediated dephosphorylation of AktS473, inhibiting Akt activity in pancreatic cells. Therefore, FKBP5 knockdown reduced PHLP levels and upregulated Akt phosphorylation [27,28]. However, the levels of AktS473 phosphorylation were not enhanced by FKBP5 downregulation in normal and cancerous tissues of mice transplanted with a melanoma xenograft [33]. Besides, FKBP5 positively regulates Akt activity in SKOV3 cells as seen by the observations that ectopic expression of FKBP5 resulted in elevated AktS473 phosphorylation, whereas FKBP5 silencing decreased S473 phosphorylation [15]. Therefore, the possibility that FKBP51 deactivates Akt may depend on specific cell types or pathways upregulated in tumors.

Apart from binding and regulating Akt, FKBP5 also interacts with the acetyltransferase P300, a transcriptional co-activator [29]. This observation suggests that FKBP5 may act as a histone chaperone, and may be involved in transcriptional regulation.

In our study, FKBP5 was not only overexpressed in taxol-resistant ovarian SKOV3 cells, but ectopic expression of FKBP5 also enhanced chemoresistance in SKOV3 cells (by 8.7 fold for taxol and 1.8 fold for vincristine). Conversely, depleting FKBP5 using short-hairpin RNA induced taxol sensitivity in a resistant SKOV3 cell subtype (i.e., SKOV3/Tx600, 8.9 fold). Although the manner in which FKBP5 is involved in regulating drug sensitivity remains unclear, it appears to represent a crucial factor in determining sensitivity to mitotoxic drugs in ovarian cancer cells. In addition, several studies indicate that FKBP51 may represent

The PI3K/Akt signaling pathway is crucial for cancer proliferation

The PI3K/Akt pathway is critical for cell survival, proliferation, and angiogenesis, and is frequently dysregulated in human cancer [12]. Accordingly, inhibition of the PI3K/Akt pathway using the PI3K inhibitors wortmannin and LY294002 inhibits cancer cell proliferation [13]. There are three major classes of PI3Ks but only class I has been heavily implicated in oncogenesis [14]. Akt is activated by PI3K, and subsequently modulates several critical substrates (such as AR, NF-kB, FOXI, transcription factors, Bcl-2-associated agonists of cell death, GSK3β and MDM2), which regulate cell survival, cell cycle progression, and expression of the multidrug resistant protein ABCB1/P-gp [14-16]. On the other hand, taxol-resistant (txr) cells established in our lab also showed enhanced Akt phosphorylation (except when ABCB1 was overexpressed). It was therefore proposed that activation of the PI3K/Akt pathway may represent an obstacle to anticancer drug treatments. Accordingly, inactivating the PI3K/Akt pathway using LY294002 sensitized taxol-resistant DU145 prostate cancer cells to taxol, possibly due to the high PI3K/Akt activity observed in these cancer cells [31]. Therefore, PI3K/Akt inhibitors could be helpful to circumvent chemoresistance, even in combination with other anticancer therapies.

Several PI3K/Akt inhibitors have been designed and investigated in clinical trials. For example, inhibition of PI3K activity using LY294002 was shown to sensitize U251 glioblastoma cell line cells to radiation [17]. Furthermore, enhanced taxol sensitivity was achieved with the Akt inhibitor MK-2206 in the SKOV3 ovarian cancer cell line [18]. In addition to the efficient inhibition produced by the Akt inhibitor on SKOV3 epithelial carcinoma, several PI3K/Akt inhibitors have shown potent antitumor activity in squamous cell lung carcinoma [12,19].

resistance may be involved in the death of more than 90% of cancer patients. Previous studies on the mechanisms of taxol resistance have identified various factors involved in this process, including tubulin mutations, tubulin isoforms, overexpression of multidrug resistance proteins, and Bcl-2 activity. The inhibitor of Bcl-2/Bcl-XL termed ABT-737 was shown to be highly efficient at enhancing sensitivity of taxol [10]. In addition, the dual Bcl-2/Mcl-1 inhibitor S1 efficiently synergized with taxol by enhancing apoptosis and taxol sensitization in chronic myelogenous leukemia [11]. However, identification of single factors involved in taxol chemoresistance, such as Tau, Bcl-2 and Mcl-1, might not be sufficient to fully explain the drug-resistant phenotype of cancer cells detected in human patients [6,7].

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an interesting target for the treatment of various cancers [32-34].

**The androgen receptor potentiates FKBP5 activity in taxol resistance**

Besides possessing PPIase and steroid receptor co-chaperone activities, FKBP5 is regulated by the androgen receptor (AR). This receptor is part of the steroid hormone receptor family, and contains a DNA-binding domain and a hormone-binding domain. The AR is activated by binding androgens in the cytoplasm, which leads to its translocation to the nucleus, where it regulates the transcription of various genes, including prostate-specific antigen (PSA). AR signaling is known to regulate the growth and development of prostate and breast cancer cells [20,[21]. The PI3K inhibitor LY294002 inhibits AR activity through phosphorylation and inactivation of GSK3β, a downstream substrate of PI3K/Akt, which results in nuclear accumulation of β-catenin. β-catenin then interacts with the AR to induce HER3 gene expression and cell proliferation, an observation which shows the importance of the association between AR and Wnt signaling pathways in driving of cancer cell growth [21]. Functional annotation of AR co-expressed genes showed involvement of this protein in cell cycle or cellular metabolism, including regulation of mitosis and mitotic metaphase/anaphase transition, glucose metabolism, and oxygen homeostasis [23].

Our preliminary results identified novel upregulated taxol-resistant (txr) genes that were positively associated with the AR and FKBP5. The AR and FKBP5 both produced positive regulation in drug resistance suggesting contribution of both proteins in this process. Pathway enrichment analysis of AR-related txr genes showed that the txr genes identified were involved in regulation of the cell cycle (H1F0), development (LCN2, SPP1, FGFR2), cellular transport (ABCB1), and immune responses (LCN2). Other studies reported that taxol therapy affects AR activity, but establishing the exact mechanisms involved in this process awaits further studies.

**ABC transporters and taxol resistance**

Multidrug resistance (MDR) represents an obstacle to cancer therapy, and is constantly associated with chemotherapy-based treatment failure. ABCB1/P-gp, a drug efflux pump, is one of the main target leading to taxol resistance [3]. Accordingly, P-gp overexpression is involved in the MDR phenotype which has been detected in various types of cancer cells. Moreover, P-gp expression can also be used to grade the pathological level of colorectal carcinoma cases, with high values being found in well differentiated tumors and low values being observed in poorly differentiated tumors [5]. A meta-analysis indicated that P-gp may not be useful to predict cancer outcome. On the other hand, elevated P-gp expression correlates with poor survival in epithelial ovarian cancer [34-36].

In our microarray analysis of taxol-resistant SKOV3 cells, several ABC efflux transporters were overexpressed more than 2 fold compared to parental cells (i.e., ABCB1, ABCG2, ABCB6, and ABCC2). Up to now, at least 48 ABC transporters have been described and classified into seven subfamilies in humans (from ABCA to ABCG) [8]. The transporters ABCB1, ABCG2, and ABCCs appear to represent the main receptors involved in MDR-cancer cells [9]. Transcriptomic profiling revealed that ABCB1 remains a major target to reduce taxol resistance [15]. To improve cancer treatment, pharmacological inhibitors of the transporter pathways described here have been studied. However, targeting the ABC transporters has produced mixed results so far due to high toxicity [22].

**Linking FKBP5/AR to taxol resistance**

We recently found that the immunophilin FKBP5 is transiently overexpressed in an ovarian cell line selected for taxol resistance. Functional analysis indicated that FKBP5 expression level can be enhanced by the AR, and that this is critical for upregulating a sub-set of genes important for taxol resistance (e.g., ABCB1) [15]. This is the first observation, to our knowledge, that the immunophilin FKBP5 is linked to cell response to taxol, at least in ovarian cancer cells. Since ABCB1 has been shown to represent an important factor for multiple drug resistance [36], the finding that the FKBP5/AR/ABCB1 axis plays a role in taxol resistance in ovarian cancer cells supports the usefulness of our strategy. Although our findings are different from those of Pei et al. who observed that FKBP5 reduces pAkt levels [37], they are consistent with the findings of Fabian et al. [38] who used mutational and pharmacological studies to show that FKBP inhibitors may not inhibit the Akt-FKBP-PHLPP pathway. Additional proteins that interact with FKBP5 may provide an additional layer of regulation, and this regulation is likely to be cell-type dependent. Our findings suggest that regulation of taxol resistance genes by FKBP5 is specific to the drug used. Identification of unique taxol resistance genes may provide important markers for the development of new cancer treatments based on single or multiple drug(s).

Taken together, our results confirm that FKBP5 acts as a scaffold protein [39] that recruits the AR and regulates gene expression. Amplification of FKBP5 expression also subsequently upregulates target taxol resistance genes such as ABCB1, H1F0, BMP5, and FGFR. However, our current data did not show whether FKBP5 can directly regulate taxol
response in ovarian cancer cells. Most of the txr genes identified are novel associations worthy of further studies. While the taxol resistance genes are highly upregulated and readily detected by transcriptomic profiling, FKBP5 may have been overlooked due to its apparent transient upregulation at the transcriptional level. Accordingly, we have identified a marker that appears to play a role in the development and maintenance of taxol resistance (Fig. 1).

**Concluding remarks and perspectives**

While the mechanism of action of transporters (e.g., ABCB1) involved in drug resistance is well known, targeting of these transporters has failed due to high toxicity \[^{122}\]. To identify drugs for second-line chemotherapy and prevent chemoresistance, it is imperative to determine the mechanisms underlying this process. Transcriptomic analysis of txr cells has identified alternative drug targets to improve treatment efficacy. The finding of novel txr genes whose expression is controlled by FKBP5 appears to be an important step in this direction.

**Conflicting interests**

The authors have declared that no competing interests exist.

**Acknowledgements**

This work was supported by grants (to C.C.-K.C) from the Ministry of Science and Technology of Taiwan (Contract No. NSC100-2320-B-182-026-MY3 and MOST103-2320-B-182-031) and Chang Gung University and Chang Gung Memorial Hospital (Contract No. CMRPD1C0191 and CMRPD190053). We apologize to colleagues whose work was not cited due to space constraints.

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