CALL FOR PAPERS | Novel Therapeutics In Renal Disease

Novel inhibitors of nuclear transport cause cell cycle arrest and decrease cyst growth in ADPKD associated with decreased CDK4 levels

Matthew Tan,1 Hiromi I. Wettersten,2 Kristy Chu,4 David L. Huso,4 Terry Watnick,5 Sharon Friedlander,6 Yosef Landesman,6 and Robert H. Weiss1,2,3,7

1 Graduate Group in Comparative Pathology, 2 Division of Nephrology, Department of Internal Medicine, and 3 Cancer Center, University of California, Davis, California; 4 Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, Maryland; 5 Division of Nephrology, University of Maryland School of Medicine, Baltimore, Maryland; 6 Karyopharm Therapeutics, Inc., Natick, Massachusetts; and 7 Medical Service, Sacramento Veterans Affairs Medical Center, Sacramento, California

Submitted 22 July 2014; accepted in final form 16 September 2014

Novel inhibitors of nuclear transport cause cell cycle arrest and decrease cyst growth in ADPKD associated with decreased CDK4 levels. Am J Physiol Renal Physiol 307: F1179–F1186, 2014. First published September 18, 2014; doi:10.1152/ajprenal.00406.2014.—Autosomal-dominant polycystic kidney disease (ADPKD) is a progressive, proliferative renal disease. Kidneys from ADPKD patients are characterized by the presence of cysts that are marked by enhanced proliferation and apoptosis of renal tubular epithelial cells. Current treatment of this disease is supportive, as there are few if any clinically validated targeted therapeutics. Given the parallels between cystic disease and cancer, and in light of our findings of the efficacy of the nuclear transport inhibitors in kidney cancer, which has similarities to ADPKD, we asked whether such inhibitors show utility in ADPKD. In this study, we tested selective inhibitors of nuclear export (SINE) in two human ADPKD cell lines and in an in vivo mouse model of ADPKD. After effective downregulation of a nuclear exporter, exportin 1 (XPO1), with KPT-330, both cell lines showed dose-dependent inhibition of cell proliferation through G0/G1 arrest associated with downregulation of CDK4, with minimal apoptosis. To analyze mechanisms of CDK4 decrease by XPO1 inhibition, localization of various XPO1 target proteins was examined, and C/EBPβ was found to be localized in the nucleus by XPO1 inhibition, resulting in an increase of CEBPβ, which activates degradation of CDK4. Furthermore, inhibition of XPO1 with the parallel inhibitor KPT-335 attenuated cyst growth in vivo in the PKD1 mutant mouse model Pkd1v/v. Thus, inhibition of nuclear export by KPT-330, which has shown no adverse effects in renal serum chemistries and urinalyses in animal models, and which is already in phase 1 trials for cancers, will be rapidly translatable to human ADPKD.

Autosomal-dominant polycystic kidney disease; exportin 1; cell cycle; cyclin-dependent kinase 4

Autosomal-dominant polycystic kidney disease (ADPKD) is the most common inherited renal disease resulting from mutations in the PKD1 (85%) or PKD2 (15%) genes, which encode polycystin (PC)-1 and -2, respectively (5). ADPKD is a relatively common disease, occurring in one out of 400 to 1,000 people without racial predilection, and accounts for ~5% of end-stage renal disease patients (22). However, despite several pipeline therapies currently being evaluated, there are as yet no specific treatments for this disease. Indeed, several therapies that have shown promise in animal models have been shown to not be translatable to human disease.

ADPKD kidneys are characterized by multiple bilateral cysts occurring in all nephron segments (20, 23). Cyst formation in ADPKD is focal, and there is evidence that a two-hit process with mutation of the wild-type allele occurs in a majority of cysts. This results in clonal expansion and growth of a population of PC-depleted cells, which ultimately results in cyst formation. Several studies have shown that there is increased proliferation of cyst-lining epithelial cells, and consistent with this property, many cancer-relevant signaling proteins have been shown to be upregulated in ADPKD kidneys, including the tyrosine kinase Src, mammalian target of rapamycin (mTOR), and serine/threonine kinase Akt (reviewed in Ref. 19). However, the full impact of such cross-pollination between oncology and nephrology in the study of this disease has yet to be realized.

Exportin 1 (XPO1) is a nuclear transporter protein whose targets include many tumor suppressor proteins, including p53 and p21; we have shown previously that inhibitors of XPO1 attenuate renal cell carcinoma (RCC) growth in vitro and in vivo through their ability to increase nuclear levels of the tumor suppressor proteins p53 and p21 (7) and thereby decrease degradation of these proteins. Given that ADPKD is characterized by upregulated cell proliferation associated with low levels of p21 (12), a cyclin kinase inhibitor whose level is regulated by PC-1 (1), we hypothesized that the XPO1 inhibitors’ ability to increase nuclear p21 would result in salutary effects in PKD cells and animal models. Here, we show beneficial effects of XPO1 inhibitors in ADPKD in vitro and in vivo. In PKD cells, treatment with an XPO1 inhibitor results in attenuation of cyclin-dependent kinase 4 (CDK4) with consequent increased C/EBPβ, cell cycle arrest in vitro, and decreased cyst growth in vivo. This mechanism of action is distinct from what has been observed in RCC. In light of the fact that phase 1 trials for the XPO1 inhibitor KPT-330 in cancer patients are currently underway (NCT01607905 and NCT01607892) and show minimal adverse effects, XPO1...
inhibition could be translated to the clinic as a novel therapeutic approach for ADPKD.

MATERIALS AND METHODS

Cell lines. WT9-7 and WT9-12 were purchased from American Type Culture Collection (Manassas, VA). WT9-7 cells are derived from proximal tubule epithelial cells, and WT9-12 cells are derived from both proximal and distal tubule epithelial cells. Cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and penicillin-streptomycin.

Materials. Lipofectamine RNAiMAX transfection reagent, Stealth RNAi negative control siRNA, and Stealth RNAi XPO1 siRNA were synthesized by Karyopharm Therapeutics (Natick, MA). Dimethyl sulfoxide (DMSO) and mouse monoclonal anti-p53 antibody [anti-rabbit IgG (H+L)] and F(ab’)2 fragment (Alexa Fluor 488 conjugate)] were obtained from Cell Signaling Technology (Beverly, MA). Goat anti-mouse and goat anti-rabbit horseradish peroxidase-conjugated IgG were obtained from Bio-Rad (Hercules, CA). Vectashield HardSet Mounting Medium with DAPI was obtained from Vector Laboratories (Burlingame, CA).

Immunoblotting. Immunoblotting was performed as described previously (6). Briefly, after appropriate treatments, the cells were washed with PBS and lysed in lysis buffer, and cell lysates were immunoblotted. Membranes were blocked in 5% nonfat dry milk for 1 h at room temperature and probed with appropriate antibodies. Membranes were then probed with horseradish peroxidase-tagged anti-mouse or anti-rabbit IgG antibodies. Signal was detected using enhanced chemiluminescence solutions. Densitometry was measured using ImageJ gel imaging software, and the relative densitometry values were calculated by dividing the density of protein of interest by the loading control (β-actin).

MTT assay. Cell viability assay was performed as described previously (6). Briefly, cells were plated in 96-well plates, and after appropriate treatments the cells were incubated in MTT solution/media mixture. Then, the MTT solution was removed and the blue crystalline precipitate in each well was dissolved in DMSO. Visible absorbance of each well at 540 nm was quantified using a microplate reader.

![Fig. 1. KPT-330 attenuated exportin 1 (XPO1) and inhibited cell viability in autosomal-dominant polycystic kidney disease (ADPKD) cells through cell cycle arrest. A: cells were treated with KPT-330 at the indicated doses for 24 h, and immunoblotting was performed. B: cells were treated with KPT-330 at the indicated doses for 16 (top) or 72 h (bottom), and an MTT assay was performed as described in MATERIALS AND METHODS. Error bars indicate standard deviation. *P < 0.05 compared with control. Results shown are representative of 3 independent experiments.](http://ajprenal.physiology.org/)

---

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Live</th>
<th>Early Apoptotic</th>
<th>Late Apoptosis/Dead</th>
<th>Debris</th>
<th>Total Apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT9-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>83.35</td>
<td>1.30</td>
<td>10.60</td>
<td>4.75</td>
<td>11.90</td>
</tr>
<tr>
<td>KPT-330 1 µM</td>
<td>87.70</td>
<td>1.25</td>
<td>7.95</td>
<td>3.10</td>
<td>9.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Live</th>
<th>Early Apoptotic</th>
<th>Late Apoptosis/Dead</th>
<th>Debris</th>
<th>Total Apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT9-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>86.20</td>
<td>3.07</td>
<td>10.24</td>
<td>0.49</td>
<td>13.30</td>
</tr>
<tr>
<td>KPT-330 1 µM</td>
<td>83.00</td>
<td>3.30</td>
<td>13.00</td>
<td>0.70</td>
<td>16.30</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Live</th>
<th>Early Apoptotic</th>
<th>Late Apoptosis/Dead</th>
<th>Debris</th>
<th>Total Apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT9-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>41.5</td>
<td>7.30</td>
<td>29.0</td>
<td>4.7</td>
<td>82.5</td>
</tr>
<tr>
<td>KPT-330 1 µM</td>
<td>42.2</td>
<td>5.70</td>
<td>26.5</td>
<td>4.6</td>
<td>80.3</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Live</th>
<th>Early Apoptotic</th>
<th>Late Apoptosis/Dead</th>
<th>Debris</th>
<th>Total Apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT9-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>40.8</td>
<td>9.20</td>
<td>25.8</td>
<td>4.7</td>
<td>80.5</td>
</tr>
<tr>
<td>KPT-330 1 µM</td>
<td>44.6</td>
<td>5.10</td>
<td>25.7</td>
<td>4.6</td>
<td>80.0</td>
</tr>
</tbody>
</table>

---

AJP-Renal Physiol • doi:10.1152/ajprenal.00406.2014 • www.ajprenal.org
Cell cycle analysis. Cell cycle analysis was performed utilizing the Muse Cell Analyzer from Millipore (Billerica, MA), following the manufacturer’s instructions. Briefly, after appropriate treatments, the cells were washed with PBS and stained with propidium iodide. After staining, the cells were processed for cell cycle analysis.

Annexin V assay. Annexin V and dead cell assay was performed utilizing the Muse Cell Analyzer from Millipore, following the manufacturer’s instructions. Briefly, after appropriate treatments, the cells were incubated with Annexin V and dead cell reagent (7-AAD), and the events for dead, late apoptotic, early apoptotic, and live cells were counted.

Immunofluorescence. After appropriate treatment in eight-well chamber slides, immunofluorescence was conducted as described previously (6). Briefly, the cells were fixed in 4% paraformaldehyde and blocked in the blocking buffer. After blocking, the cells were incubated with appropriate antibody, incubated with anti-rabbit IgG (H + L) and F(ab′)2 fragment (Alexa Fluor 488 Conjugate) and coverslipped with Vectashield with DAPI. The specimens were examined by confocal microscopy.

siRNA transfection. Lipofectamine RNAiMAX transfection reagent was mixed with Stealth RNAi siRNA, following the manufacturer’s instructions. Then cells were incubated with the mixture in the growth medium without penicillin-streptomycin for appropriate time for transfection of siRNAs.

Immunohistochemistry. A Biogenex 16000 automated immunostainer was used to carry out immunohistochemistry on formalin-fixed paraffin embedded xenografts. Antigen was retrieved by steaming with Cell Marque Declere reagent. Background was blocked with Biogenex power block. Primary antibody was applied for 1 h at room temperature, followed by detection with the two-step, Hi-Def Polymer Detection kit from Cell Marque, followed by Cell Marque DAB chromagen. Samples were counterstained with hematoxylin, dehydrated, cleared, coverslipped, and incubated with the the antibodies p53 (Vector) and Ki-67 (Cell Marque).

Pkd1v/v mouse study. All mouse experimental procedures were carried out in compliance with guidelines provided by the Office of Laboratory Animal Welfare at the National Institutes of Health (NIH), and the protocol was approved by the Johns Hopkins University Animal Care and Use Committee. The Pkd1v/v mouse model has been described previously (28). Briefly, a mutation disrupting the G protein-coupled receptor proteolytic site cleavage site encoded in PKD1 was introduced by gene targeting into the PKD1 locus. Pkd1v/v homozygous mice are born with noncystic kidneys but rapidly develop renal cysts during the early postnatal period. For treatment studies, Pkd1v/v pups were treated with KPT-335 or vehicle by intraperitoneal injection biweekly starting at P3 and P4 (postnatal days 3 and 4). The pups were weighed before each injection and received a weight-adjusted dosage of 5 mg/kg or a matching volume of vehicle. At 2 wk of age, pups were euthanized and kidneys collected for assessment and determination of kidney weight/body weight ratio for each individual as well as for appropriate staining.

RESULTS

KPT-330 attenuates XPO1 and inhibits ADPKD cell growth through G0/G1 cell cycle arrest. To test the efficacy of KPT-330 in ADPKD, we first asked whether KPT-330 attenuates levels of its target protein XPO1 [such as occurred in RCC cells (7)] and whether it attenuates [cell growth in two human
ADPKD cell lines. After incubation with KPT-330, both ADPKD cell lines (WT9-7 and WT9-12) showed decreased XPO1 protein levels at 24 h (Fig. 1A) and attenuated cell viability as assessed by MTT assay at 16 and 72 h (Fig. 1B) (since KPT-330 is a competitive inhibitor of XPO1, levels of XPO1 do not necessarily correlate with its effects on cell viability).

To further evaluate the mechanism by which cell viability was decreased by KPT-330, ADPKD cells were subjected to cell cycle and apoptosis analyses 16 h after incubation with KPT-330. Although KPT-330 did not increase the rate of apoptotic cells (Fig. 1C), it caused cell cycle arrest at the G0/G1 phase in both WT9-7 and WT9-12 cells (Fig. 1D), suggesting that the decrease in cell viability observed by the MTT assay was via cell cycle inhibition.

**KPT-330 did not affect localization of p53 and p21.** Our prior data showed that several tumor suppressor proteins are increased in RCC cell lines that are derived from renal tubule epithelial cells (7). In these cell lines, the tumor suppressor proteins are increased via enhanced nuclear localization, and treatment with KPT-330 results in increased levels of p53 and p21. To determine whether a similar mechanism might account for G0/G1 phase arrest in ADPKD cells, we incubated WT9-12 cells with KPT-330 for 24 h, and p21 and p53 subcellular was evaluated by immunofluorescence. Surprisingly, in these cells, KPT-330 did not affect localization of either p53 or p21 even at a high dose (10 µM; Fig. 2A), and immunoblotting did not show increased levels of p21 contrary to what was observed in RCC (Fig. 2B). p53 was upregulated at both concentrations tested (Fig. 2B), a finding that was most likely due to an indirect and nonspecific stress response that is a well-known stimulus for p53 transcription (11). These findings suggest that the mechanism of XPO1 inhibition attenuating cell growth in ADPKD is distinct from that which occurs in RCC cells; the mechanism of the p21 decrease in response to KPT-330 in these cells was not investigated in the current study.

**KPT-330 attenuated CDK4 protein levels through upregulation of C/EBPα and nuclear localization of C/EBPβ.** To ascertain a mechanism for the G0/G1 phase arrest observed in ADPKD cells, we next evaluated a number of signaling proteins known to be involved in the G0/G1 phase transition. Of several such proteins screened, we observed that CDK4 levels were attenuated by KPT-251 (an earlier generation of the XPO1 inhibitor) in both ADPKD cell lines, whereas cyclin-D1 levels were not affected by XPO1 inhibition (Fig. 3A). This CDK4 level change was validated in a dose-dependent experiment using KPT-330 (Fig. 3B), suggesting the possibility that CDK4 decrease plays a role in the G0/G1 arrest by XPO1 inhibition. To confirm that CDK4 attenuation is due to XPO1 inhibition and was not an off-target effect of KPT-251 and -330, we showed that CDK4 levels (and as a confirmation, XPO1 levels) were attenuated by transfection of the cells with an siRNA specific to XPO1 (Fig. 3C).

Since CDK4 has not been shown to be an XPO1 “cargo” protein [i.e., one of the proteins that is transported from the nucleus to the cytosol by the selective inhibitors of nuclear export (SINE)], we next asked whether there are known XPO1 cargoes that interact with CDK4 and could thus mediate its downregulation. C/EBPα has been reported to attenuate CDK4 protein levels via increased degradation (24, 25). Since C/EBPβ, one of the transcription factors of C/EBPα (21), is a known cargo protein of XPO1 (17), we hypothesized that XPO1 inhibition causes nuclear confinement of C/EBPβ, resulting in upregulation of C/EBPα and subsequent degradation of CDK4. To test this hypothesis, we evaluated subcellular

![Fig. 3](http://ajprenal.physiology.org/)
localization change of C/EBPβ induced by KPT-330 and consequent changes in levels of C/EBPα. When treated with KPT-330, C/EBPβ was confined to the nucleus, as assessed by immunofluorescence (white arrows; Fig. 4A), and level of C/EBPα was increased in WT9-12 cells (Fig. 4C), suggesting that the attenuation of CDK4 by XPO1 inhibition is through nuclear confinement of C/EBPβ and consequent transcriptional upregulation of C/EBPα. Although in WT9-7 cells C/EBPβ was also confined to the nucleus (Fig. 4A), C/EBPα protein levels as evaluated by immunoblotting gave ambiguous results.

XPO1 inhibition attenuates ADPKD cyst growth in vivo. As a next step toward translating XPO1 inhibition to ADPKD clinical trials, we evaluated the efficacy of an intraperitoneally administered XPO1 inhibitor, KPT-335, in the Pkd1v/v model mouse (28). Briefly, since these mice rapidly develop cysts when the animals are too small to perform oral gavage, KPT-335 was administered intraperitoneally in these animals (control, n = 7; KPT-335, n = 5). Mice were treated three to four times beginning at P3 and P4 and euthanized 2 wk after birth. The total kidney weight/body weight ratio (15, 28) was significantly lower in KPT-335 vs. control vehicle-treated animals (Fig. 5A). To determine whether the decreased kidney sizes resulted from induction of cell cycle arrest as seen in vitro, we tested these kidneys for the expression of the proliferation marker Ki-67 by immunohistochemistry (Fig. 5B). Interestingly, a clear reduction in Ki-67 was observed in treated kidneys vs. controls, suggesting that treatment with KPT-335 led to inhibition of proliferation.

There was no obvious change between treated and control kidneys in cyst size or morphology in the sections of kidney evaluated, which may have been due to a selection bias. However, as the mTOR inhibition study has shown, kidney size (reflecting cyst size) and renal function are not always correlated (reviewed in Ref. 9).

Although we were unable to get sufficient biofluids from these 2-wk-old animals for analysis of kidney function, toxicology studies with KPT-330 (the orally bioavailable XPO1 inhibitor that is currently in use in phase 1 trials for cancers) showed no increases in serum BUN or creatinine and no changes in serum bicarbonate or in dipstick proteinuria after 4 and 13 wk of treatment in wild-type Sprague-Dawley rats and cynomolgus monkeys (data not shown). To assess on-target effects of KPT-335 in vivo in the Pkd1v/v mice, we performed immunohistochemical staining for the XPO1 cargo protein DMSO

Fig. 4. KPT-330 confined C/EBPβ in the nucleus and increased C/EBPα levels in ADPKD cells. A: cells were treated with DMSO or KPT-330 at 1 µM for 24 h, and immunofluorescence was performed. B: the number of C/EBPβ (white arrows) that were predominantly in the nucleus were counted in 3 randomly selected fields and divided by the total cell number. Blue, nucleus (DAPI); green, C/EBPβ. *P < 0.05 compared with control. Error bars indicate SD. C: cells were treated with DMSO or KPT-330 at 1 µM for 24 h, and immunoblotting was performed.
p53. p53 showed clear confinement within the nuclei of tubular epithelial cells in the treatment group, confirming on-target effects of the XPO1 inhibitor in the kidneys of these animals.

DISCUSSION

ADPKD is the most common monogenic cause of renal disease in the US, and it is responsible for renal failure in 5% of end-stage renal disease patients (22). Although various potential targets, including Src, p21, and Akt/mTOR, have been identified in various rodent models of cystic disease (12, 13, 20), none has yet been proven to be translatable to human disease. XPO1 is a nuclear exporter protein that functions to transport leucine-rich nuclear proteins, as well as some mRNAs and microRNAs, from the nucleus to the cytosol (3, 8, 10, 18). Studies in various cancers have shown that by using XPO1 inhibitors, tumor suppressor proteins can be retained in the nucleus to induce cell apoptosis (7, 14, 16) and cell cycle arrest (7). Since PKD has been found to have parallels to neoplasia (4), and because we have shown previously that the cyclin kinase inhibitor p21, whose level is regulated by PC-1 (1), is decreased in PKD, the concept of evaluating this particular cancer therapeutic for ADPKD seemed logical.

We found that the XPO1 inhibitor KPT-330 caused cell cycle arrest rather than apoptosis in PKD cells in vitro. Interestingly, KPT-330 showed efficacy starting at 1 μM in ADPKD cells, which is an order of magnitude lower than the effective dose in normal human renal tubular epithelial cells (10 μM; Wettersten H and Weiss RH, unpublished observations), suggesting minimal if any renal toxicity by KPT-330 when brought to the bedside. While the cell cycle was found to be inhibited specifically in the G0/G1 phase, likely through a downregulation of CDK4 protein in vitro and in vivo, KPT-330 did not result in significant apoptosis of ADPKD cells. This contrast with cancer cell studies (2, 7, 29) highlights a fundamental difference in apoptosis signaling between the two diseases: increased apoptosis has been demonstrated to occur in ADPKD (4, 27), whereas decreased apoptosis is a hallmark of malignancy. Thus, our findings suggest that treatment with SINE in ADPKD cells does not produce an observable additive effect to an already highly apoptotic environment.
Although we did not find alterations of p21 and p53 localization with inhibition of XPO1 in ADPKD, which would have been predicted from our observations in RCC, we did observe that a key G1/S phase regulatory protein, CDK4 (26), was consistently downregulated by XPO1 inhibition in ADPKD cells and is thus a likely mechanism for its effect. However, since CDK4 is not a known cargo protein of XPO1, we evaluated known XPO1 cargos and identified C/EBPβ as the likely mechanistic link with CDK4 via its nuclear localization and subsequent upregulation of C/EBPα. Other studies have identified similar mechanisms: work on adipogenesis by ceramide showed that the inhibition of XPO1 was able to reverse the effect of ceramide-induced decrease of C/EBPβ nuclear localization (17). C/EBPβ transcriptionally activates the C/EBPα gene through a C/EBP regulatory binding site (21), and consequently, C/EBPα promotes proteasome-dependent degradation of CDK4 through CDK4-ubiquitin conjugation (24). To further support this mechanism specifically with XPO1 inhibitors, another study has shown that C/EBPα protein levels are upregulated through XPO1 inhibition by SINE (13a). Taken together with our data, it is likely that KPT-330 decreased CDK4 protein levels through nuclear localization of C/EBPβ, resulting in upregulation of C/EBPα.

The mechanism of KPT-330 attenuating ADPKD cell growth was distinct from that in RCC cells. In our previous SINE studies in RCC (7), the SINEs KPT-185 and -251 confined p53 and p21 in the nucleus, causing both cell cycle arrest and apoptosis, but in the current study, p53 and p21 localization were unaffected by KPT-330 in ADPKD cells. These findings suggest that, although there are indeed parallels between ADPKD and RCC, there are also significant differences in signaling pathways that are most likely related to differential apoptotic phenotypes and to the deranged ciliary signaling seen in cystic disease but not described in cancer.

We further confirm a salutary effect of XPO1 inhibition in vivo via an intraperitoneally administrable SINE, KPT-335. We observed a significant decrease in total kidney/body weight ratio in the KPT-335-treated group compared with vehicle, which implied that KPT-335 was inhibiting cyst growth in the kidneys. In addition, we found Ki-67 to be downregulated in the KPT-335-treated mouse kidney tissues, which indicates that inhibition of XPO1 resulted in cell cycle arrest, as was shown in our in vitro results. In light of earlier data in rats and monkeys showing no evidence of renal toxicity or acid base derangements, further studies in our laboratory with these compounds will be focused on adult-onset PKD animal models.

CONCLUSION

Treatment of ADPKD cells with specific inhibitors of XPO1 resulted in G0/G1 cell cycle arrest through a cascade of events that involves attenuation of CDK4 and the activities of C/EBPα and C/EBPβ, and the efficacy of KPT-335 was confirmed in the PKD1 mutation mouse model showing attenuation of cyst growth by XPO1 inhibition. In this study, we have introduced a novel treatment paradigm for treatment of ADPKD with inhibitors of XPO1, which bodes further animal investigation and subsequent human trials.

GRANTS

This work was supported by NIH Grants 5-U01-CA-86402 (Early Detection Research Network), 1-R01-CA-135401-01A1, and 1-R01- DK-082690-01A1, the Medical Service of the US Department of Veterans Affairs, and the Clayton Foundation (to R.H. Weiss), the LNL-UCDCC Fitzpatrick Award (to H. I. Wettersten) and NIH Grant R01-DK-095036 (to T. Watnick). These studies utilized reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases-sponsored Baltimore Polycystic Kidney Disease Research and Clinical Core Center (P30-DK-09068).

DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS


REFERENCES