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KPT-330 inhibitor of XPO1-mediated nuclear export has anti-proliferative activity in hepatocellular carcinoma

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Abstract

PURPOSE—Exportin-1 (XPO1, CRM1) mediates the nuclear export of several key growth regulatory and tumor suppressor proteins. Cancer cells often overexpress XPO1 resulting in cytoplasmic mislocalization and aberrant activity of its target proteins. Orally bioavailable selective inhibitors of nuclear export (SINE) that irreversibly bind to and inhibit the function of XPO1 have been recently developed. The aim of this study was to investigate the efficacy of the clinical staged, orally available, SINE compound, KPT-330 in Hepatocellular carcinoma (HCC).

METHODS—In silico meta-analysis showed that *XPO1* is overexpressed in HCC. Six HCC cell lines were treated with KPT-330 and cell proliferation and expression of cell growth regulators were examined by cell proliferation assays and Western blot analysis, respectively. The in vivo anti-cancer activity of KPT-330 was examined in a HCC xenograft murine model.

RESULTS—KPT-330 reduced the viability of HCC cell lines in vitro and this anti-proliferative effect was associated with cell cycle arrest and induction of apoptosis. The expression of the pro-apoptotic protein PUMA was markedly up-regulated by KPT-330. In addition, SINE treatment increased the expression of the tumor suppressor proteins p53 and p27, while it reduced the expression of HCC promoting proteins, c-Myc and c-Met. XPO1 levels itself were also down-regulated following KPT-330 treatment. Finally, a HCC xenograft murine model showed that treatment of mice with oral KPT-330 significantly inhibited tumor growth with little evidence of toxicity.

CONCLUSION—Our results suggest that SINE compounds, such as KPT-330 are promising novel drugs for the targeted therapy of HCC.

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Conflict-of-interest

Disclosure: S.S. and M.K. are employees of Karyopharm Therapeutics, a clinical stage biopharmaceutical company that develops selective inhibitors of nuclear export-targeted therapeutics. The remaining authors declare no competing financial interests.

Keywords

hepatocellular carcinoma; XPO1 inhibitor; apoptosis; xenograft

Introduction

Liver cancer is one of the most common cancers worldwide and the third most common cause of cancer-related mortality [1–3]. Both the incidence and mortality of liver cancer are rising. Over 80% of liver cancer cases are classified as hepatocellular carcinoma (HCC), characterized by late-stage diagnosis and a poor prognosis. For most patients with advanced stages of the disease, therapeutic options are still inadequate. Currently, Sorafenib, a multikinase inhibitor is the only targeted therapeutic drug approved for treatment of advanced HCC [4]. However, too often patients either fail initial sorafenib therapy or develop resistance after initial response. Identification of new drugs targeting different cellular pathways is clearly needed.

Transport between the nucleus and the cytoplasm is a fundamental process of eukaryotic cells. Exportin-1 (XPO1, CRM1) is one of several nuclear export receptors, which is essential for nuclear export of numerous proteins as well as RNA molecules [5,6]. Interestingly, XPO1 is the sole nuclear exporter responsible for the transport of the majority of major tumor suppressors and growth regulatory proteins, such as p53, p27, FOXO1, IκB, cyclin B1, Cyclin D1 and Survivin [7,8]. XPO1 expression levels are up-regulated in a number of solid tumors (glioblastoma, ovarian, pancreatic and cervical cancers) [9–12], and haematological malignancies (acute myeloid leukemia [AML], chronic lymphocytic leukemia [CLL], Non-Hodgkin's lymphomas [NHL], multiple myeloma [MM], chronic myelogenous leukemia [CML] and B-cell acute lymphoblastic leukemia [B-ALL]) [13–18]. The increase in XPO1 levels leads to miss-localization of tumor suppressors and cell cycle regulators which in turn results in their inactivation or aberrant activation. Indeed, overexpression of XPO1 is associated with poor prognosis and resistance to chemotherapy. Inhibition of XPO1was therefore, proposed as an attractive antineoplastic therapeutic strategy. However, initial XPO1 inhibitors, of which leptomycin B (LMB) is the most well-known, showed limited clinical applicability due to severe toxicity and minimal efficacy potentially due to off target activity [7,8,19,20].

XPO1 binds to its cargo proteins by recognizing a hydrophobic, leucine-rich nuclear export signal (NES) [21,22]. Recently, a new class of potent and slowly reversible small-molecule covalent inhibitors of XPO1, known as Selective Inhibitors of Nuclear Export (SINE), were developed (Karyopharm Therapeutics Inc.). SINE selectively bind to Cys528 located in the NES-binding groove of XPO1, thereby inhibiting XPO1 binding to its target proteins [17,23,24]. These compounds exert anti-tumor activity in several malignances in vitro as well as in animal models [13,18]. Importantly, SINE showed minimal toxicities in normal cells. Based on these promising results, phase one clinical trials of the SINE compound, KPT-330 are ongoing in patients with either relapsed or refractory hematologic malignancies (NCT01607892) and solid tumors (NCT01607905, NCT01896505). In the

current study, we investigated the anti-cancer potential of SINE against HCC cell lines and in a HCC xenograft in vivo model.

Martials and Methods

Cell lines and reagents

Hepatocellular carcinoma cell lines, SK-HEP-1, Huh7 and MHCC97H, were kindly provided by Dr. X. Chen (UCSF, San Francisco); SNU-182 and SNU-387 were kindly provided by the National University of Singapore. HepG2 was obtained from ATCC. All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen Life Technologies). XPO1inhibitors included LMB (Sigma-Aldrich) as well asKPT-185 and KPT-330 (Karyopharm Therapeutics). For in vitro studies, inhibitors were dissolved in DMSO. For the in vivo study, KPT-330 was dissolved in vehicle solution ([0.6% (w/v] Pluronic F-68 and 0.6% [w/v] PVP-K29/32 in sterile water).

Cell proliferation, cell cycle analysis apoptosis assays and measurement of mitochondrial membrane potential

Cellular proliferation was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich) following the manufacturer's instructions. Cells were plated at a density of 1,000 to 3,000 cells per well in 96-well plates and incubated with either vehicle (DMSO) or increasing concentrations of XPO1 inhibitors. Cell proliferation was measured 72 hrs later and reported as a percentage of DMSO control cells. IC_{50} values represent the concentration at which cell growth was inhibited by 50%. For cell cycle analysis, cells were cultured with either diluent control (DMSO) or various concentrations of KPT-330 for 24 hrs, washed and fixed in 70% ethanol. Cells were stained with propidium iodide (PI)/RNase and analyzed by flow cytometry using Becton Dickinson FACScan flow cytometer. For apoptosis analysis, cells were cultured with DMSO or various concentrations of KPT-330 for 72 hrs. Apoptosis was measured using Annexin V-FITC Apoptosis Detection Kit (BD Pharmingen) according to the manufacturer's instructions. Mitochondrial membrane potential depolarization was assessed by JC-1 assay Kit (M34152 MitoProbe, Life technologies) according to the manufacturer's instructions. Samples were analyzed by flow cytometer (CyAn™ ADP Analyzer, Beckman Coulter).

Western blot analysis

Cell lysates were prepared using lysis buffer [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 0.5% NP-40] containing protease and phosphatase inhibitor cocktails (Roche Molecular Biochemicals). Protein samples (50 μg) were subsequently separated on 4–15% gradient SDS-polyacrylamide gels (Bio-Rad) and transferred to polyvinylidene difluoride membranes (Immobilion, Millipore). SuperSignal West Pico and West Dura Chemiluminescent substrates (Pierce Biotechnology, Rockford, IL, USA) were used for protein detection. The following primary antibodies were used: anti-XPO1 (BD Biosciences);anti-p53 (DO-1), anti-BCL2, anti-BAX, anti-PUMA, anti-p27, anti-c-Myc, anti-c-Met(Santa Cruz Biotechnology, Santa Cruz, CA);and anti–β-actin (Sigma-Aldrich).

HCC xenograft murine model

All animal experiments were conducted in accordance with the guidelines of Cedars-Sinai Research Institute and the National Institute of Health. The protocol was approved by the Animal Care and Use Committee at Cedars-Sinai Medical Center Institution (protocol number IACUC004273) and all efforts were made to minimize animal suffering. Six-week-old female nu/nu athymic nude mice were injected subcutaneously with SK-HEP-1 (5×10^6) cells suspended in 0.15 mL Matrigel (Basement Membrane Matrix, High Concentration; BD Biosciences). Five mice were randomly assigned to each experimental group: (1) vehicle control (Pluronic F-68/PVP-K29/32); (2) low-dose KPT-330 (10 mg/kg); (3) high-dose KPT-330 (20 mg/kg). Treatments were initiated one day after cell implantation, and were given by oral gavage 3 times a week on non-consecutive days for 4 weeks. Tumor volume was measured by calipers thrice weekly and calculated using the formula: (length × width × depth)/2. All mice were observed for changes in body weight and possible side-effects. Animals were euthanized on day 33. The tumors were dissected, and either fixed in 10% formalin, or snap frozen in liquid nitrogen followed by homogenizing on ice for western blotting.

Statistical analysis

Statistical significance was determined by Student's *t*-test (two-tailed) and *P* values <0.05 were considered statistically significant.

Results

XPO1 inhibition suppresses proliferation of HCC cells in vitro

Analysis of *XPO1* expression levels in gene expression microarray studies performed on large cohorts of HCC patient samples (accession numbers GSE6764, GSE14520, GSE3500 and GSE14323, available at http://www.ncbi.nlm.nih.gov/geo/), as well as in The Cancer Genome Atlas (TCGA) revealed that *XPO1*is overexpressed in HCC compared to normal liver tissue (Table 1, Supplementary Fig. 1). In addition, Western blot analysis revealed prominent XPO1 expression in HCC cell lines (Supplementary Fig. 2). We, therefore, reasoned that inhibition of XPO1may have anti-tumor activity in HCC. The effect of the SINE XPO1inhibitors KPT-276 and KPT-330 on HCC cell viability was evaluated and compared with that of LMB. A panel of six hepatic cancer cell lines (SK-HEP-1, HepG2, Huh7, SNU-387, SNU-182, and MHCC97H) was incubated with increasing concentrations of either LMB, KPT-276 or KPT-330 for 72 hrs. All three inhibitors induced significant dose-dependent growth inhibition as measured by MTT assays (Fig. 1). The estimated IC_{50} values ranged from 0.4 nM to 12.6 nM for LMB, from 49.9 nM to 708.3 nM for KPT-276 and from 21.7 nM to 448.0 nM for KPT-330 (Table 2). Overall, growth inhibition induced by the SINE compounds, as well as LMB was more prominent in p53 wild-type cells (HepG2 and SK-HEP-1) than in p53-mutant cells (Huh7, SNU-387, SNU-182 and MHCC97H; Huh7 cells harbor a p53 mutation, yet these cells maintain p53 transcription activity [25], which may in part explain their high sensitivity to KPT-276 and KPT-330).

KPT-330 induces cell cycle arrest and apoptosis in HCC cell lines

To determine whether the observed decrease in cell proliferation is due to cell cycle arrest and/or apoptosis, we measured the effect of KPT-330 on cell cycle progression and apoptotic response in two HCC lines, SK-HEP-1 (p53 wild-type) and SNU-387 (p53 mutant). Cells were treated with either increasing concentrations of KPT-330 (SK-HEP-1, 20–2000 nM; SNU-387, 10–1000 nM) or diluent control for 24 hrs and cell cycle distributions were determined by PI staining and flow cytometry (Fig. 2A). Upon incubation with KPT-330, SK-HEP-1 cells showed a dose-dependent accumulation in both G1 and G2/M, indicating cell cycle arrest in both of these phases. On the other hand, SNU-387 cells only showed increased fraction of cells in G2/M, and not in G1.

To measure the apoptotic response, cells were co-stained with Annexin V and PI to monitor early apoptosis, as well as late-apoptosis and/or necrosis. After 72 hrs exposure to KPT-330, both SK-HEP-1 (200 nM) and SNU-387 (300 nM) showed an increase in early, as well as late apoptosis (Fig. 2B). Western blot analysis showed that following KPT-330 treatment, levels of PUMA (a pro-apoptotic member of the BCL2 family member) were slightly up-regulated in SK-HEP-1 cells and were dramatically up-regulated in two other HCC cell lines, HepG2 and MHCC97H (Fig. 2C). The levels of the anti-apoptotic protein BCL2 remained unchanged upon KPT-330 treatment while levels of the pro-apoptotic BCL2 member, BAX were up-regulated only in MHCC97H cells.

To analyze further the effect of KPT-330 on the apoptotic response in HCC cells, we measured loss of mitochondrial membrane potential, a hallmark of apoptosis. JC-1 is a cationic dye that accumulates in mitochondria. Under normal conditions, JC-1 exists as dimers and accumulates as aggregates in the mitochondria that fluoresce in the red range. In apoptotic and necrotic cells, JC-1 exists in the cytosol as monomers that fluoresce in the green range. SK-HEP-1 and Huh7 cells were treated with increasing consternations of KPT-330 for 48 hrs and changes in mitochondrial membrane potential were analyzed by FACS after JC-1 staining. Results showed a dose-dependent increase in green fluorescence, indicating loss of mitochondrial membrane potential following KPT-330 treatment (Fig. 2D).

KPT-330 treatment modulates levels of tumor suppressor and oncogenes

To begin evaluating the cellular pathways altered by SINE in HCC cells, we next analyzed the effect of KPT-330 on the protein expression levels of two well-known tumor suppressor XPO1 cargo proteins, p53 and p27 as well as c-Myc and c-Met, two oncogenes with important roles in HCC [3], and XPO1 itself. SK-HEP-1, HepG2 and MHCC97H cells were treated with increasing concentrations of KPT-330 and protein expression was analyzed by western blot (Fig 3). Following KPT-330 treatment, a significant dose-dependent up-regulation of p53 was noted in SK-HEP-1 and HepG2 cells (p53 wild type); while up-regulation of p27 occurred in all three cell lines. c-Myc level markedly decreased in SK-HEP-1 and HepG2 cells; however, no significant change in c-Myc expression occurred in MHCC97H. Levels of c-Met and XPO1 were down-regulated in all three cell lines.

KPT-330 significantly inhibits growth of HCC cells in a xenograft model

To test if XPO1 inhibition affects HCC development in vivo, a xenograft model using SK-HEP-1 HCC cells was established. SK-HEP-1 tumor cells were subcutaneously implanted in athymic nude mice, which were administered either vehicle control or one of two KPT-330 doses (low-dose, 10 mg/kg or high dose, 20 mg/kg) by oral gavage 3 days a week. The experiment ended on day 33 due to excessive tumor size in the vehicle-treated control group. Both low and high doses of KPT-330 significantly ($P<0.001$ for both doses) inhibited tumor growth (Fig. 4A and C). At the beginning of the study, moderate loss in body weight (≤ 10%), suggestive of drug toxicity, was observed in the group treated with high dose KPT-330, but this was partly reversed by the end of the study and did not appear adversely to affect the mice (Fig. 4B). Concordantly with our in vitro results, western blot analysis of tumor tissue showed that the protein levels of XPO1 were lower in tumors from KPT-330 treated mice compared with tumors from control mice (Fig. 4D).

Discussion

The present findings establish the anti-cancer effects of the clinical stage, orally available SINE XPO1 inhibitor, KPT-330, against liver cancer cells, both in vitro and in a xenograft model. Using publicly available data bases, we found that *XPO1* is overexpressed in HCC, suggesting that XPO1 may be a therapeutic target in HCC. Our data show that nanomolar concentrations of KPT-330, the first clinically tested SINE compound, leads to growth arrest and apoptosis in six HCC cell lines and suppresses growth of SK-HEP-1 HCC cells in immunocompromised mice with very little toxicity.

XPO1 facilitates the nuclear export of over 200 proteins [26,27]. Among XPO1 cargo proteins are key mediators of proliferative signaling pathways, hence, XPO1 is critical for the survival of cancer cells [7,8]. Yet, the pathways responsible for anti-proliferative effects induced by SINE are not well characterized. A previous study found that p53 status was a major factor determining the apoptotic response to KPT-185 in AML cell lines and primary cells [15]. However, inhibition of proliferation by SINE in AML is p53-independent [15,23]. Likewise SINE exhibit p53-independent anti-cancer activity in NHL [17], MM [14] and pancreatic cancer cells [24], potentially through enhancement of p73 and p27 pathways. In our study, KPT-330 reduced proliferation in both p53 wild-type and p53 mutant HCC cells, although the affect was more prominent in p53 wild-type cells. KPT-330 antiproliferative effects in HCC cells were associated with cell cycle arrest and induction of apoptosis. The apoptotic response in HCC cells was accompanied by a dramatic decrease in mitochondrial membrane potential. SINE compounds have been shown to be highly effective in inducing apoptosis in several types of transformed cells, while counterpart normal cells were shown to be much more resistant. Our finding that KPT-330 decreases mitochondrial membrane potential is consistent with a number of earlier studies pointing to the involvement of the intrinsic (mitochondrial) signaling pathway in SINE-induced apoptosis. For example, KPT-185 induced the expression of the BCL2 family members PUMA and BAX in AML and multiple myeloma cells [14,15]. In other reports, overexpression of the anti-apoptotic protein BCL2 in SINE-sensitive AML and T-ALL cell lines suppressed KPT-185 and KPT-330 induced apoptosis [28]. And finally, a combination of a BCL2 inhibitor with

KPT-185 had significant synergistic cytotoxicity in non-small cell lung cancer cells that were otherwise resistance to SINE [29]. p53 is directly involved in the intrinsic apoptosis pathway by interacting with BCL2 family members to induce mitochondrial outer membrane permeabilization [30]. We found that KPT-330 treatment resulted in loss of mitochondrial membrane potential in both p53 wild-type and p53 mutant cells, suggesting that p53 mitochondrial activity may not be essential for KPT-330-induced apoptosis in HCC cells. Among several BCL2 family members we tested, induction of PUMA by KPT-330 appeared to be the most prominent in the HCC cells. Interestingly, recent studies suggested that sorafenib induces apoptosis in HCC through an intrinsic mechanism where up-regulation of PUMA in a p53-independent manner, plays an essential role [31,32]. Taken together with our data, these results suggest that PUMA may play an important common role in mediating apoptotic death of HCC cells in response to targeted therapies.

SINE causes nuclear retention and up-regulation of various tumor suppressors including FOXO, p21, p27, IκB and p73 [13,14,17]; on the other hand, SINE compounds induce a reduction in the levels of molecules associated with cancer cell proliferation such as c-Myc [14,16]. We found that treatment of HCC cell lines with KPT-330 increased levels of p53 and p27, and reduced levels of XPO1, c-Myc and c-Met. Our observation that XPO1 levels decreased after KPT-330 treatment is in agreement with a pervious study showing that KPT-185 reduced XPO1 protein levels in AML [33]. How inhibition of XPO1 leads to its down-regulation is currently unknown. A study using a different XPO1 inhibitor (CBS9106) also noted decreased levels of XPO1 following XPO1 inhibition, and suggested that depletion of XPO1 by CBS9106, required the ubiquitin/proteasome pathway [34].

The transcription factor c-Myc and the tyrosine kinase receptor c-Met are frequently overexpressed and play tumor-promoting roles in HCC [2,3]. c-Myc and c-Met are not XPO1 target proteins; and it is not clear how XPO1 inhibition leads to reduction in their expression levels. Of note, reduced c-Myc levels following SINE treatment was also reported in MM [14,16]. Also, similar to our observation that KPT-330 down-regulated the c-Met tyrosine kinase receptor in HCC cells, KPT-185 down-regulated the oncogenic tyrosine kinase receptors FLT3 and c-KIT in AML [33]. Collectively, these data support a model in which SINE inhibition of XPO1 suppresses cancer cells both by leading to nuclear retention/activation of tumor suppressors and by inducing down-regulation of oncogenic proteins. Given that malignant cells are invariably dependent on abnormal activation of oncogenes and/or inactivation of tumor suppressor genes, this duel effect may provide the basis for the ability of SINE to kill cancer cells while sparing normal cells.

In an era of personalized cancer therapies, in which targeted drugs are often developed against specific driver mutations or tumor suppressors, XPO1 inhibition by SINE appears to be fairly broad without requiring a particular tumor type or cancer cell genotype to be effective. Consequently, SINE show robust anti-cancer activity in preclinical models for a growing list of hematologic malignancies and solid tumors. Our results suggest that SINE XPO1 antagonists are promising clinical candidates for HCC, a disease for which current treatment options are severely limited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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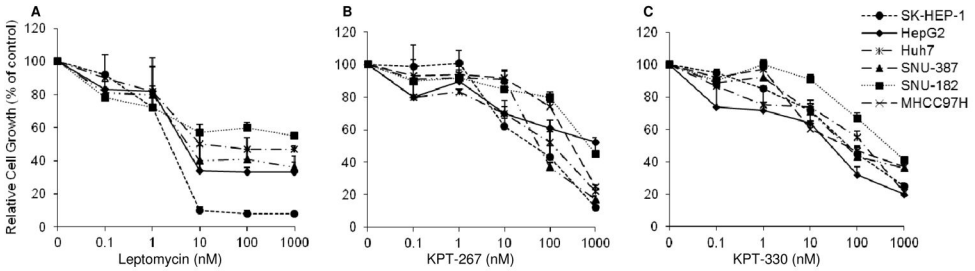

Figure 1. SINE significantly inhibit proliferation of HCC cell lines

HCC cell lines were treated with either vehicle (DMSO) or increasing concentrations of either LMB (**A**), KPT-276 (**B**) or KPT-330 (**C**) for 72 hrs, and cell proliferation was measured by MTT assays. Results represent the mean ± SD of three independent experiments performed in triplicates.

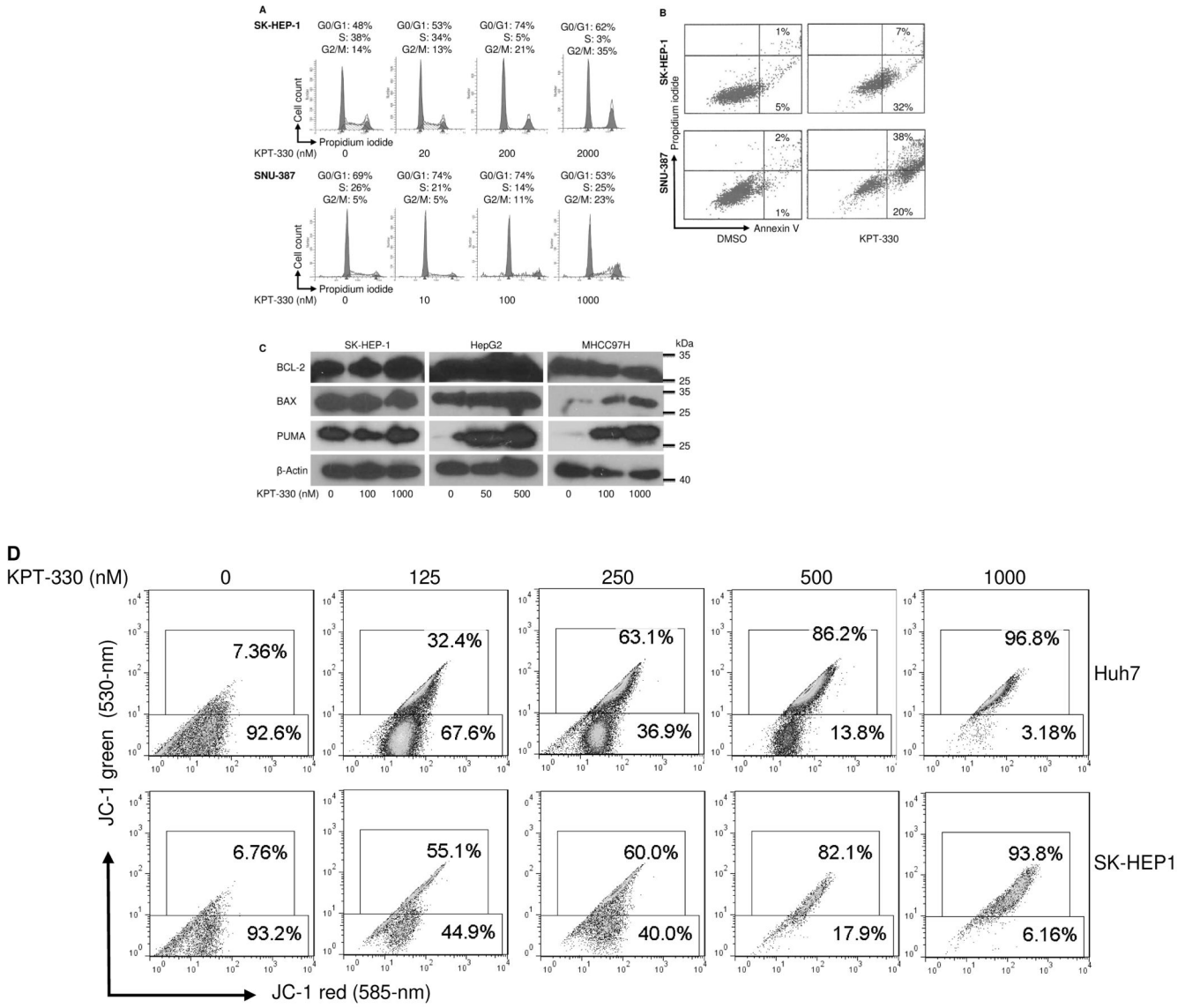

Figure 2. KPT-330 induces cell cycle arrest and apoptosis in HCC cell lines

A. SK-HEP-1 and SNU-387 cells were treated with either diluent control (DMSO) or increasing concentrations of KPT-330 for 24 hrs, stained and analyzed by flow cytometry. Propidium iodide (DNA content) histograms are shown; one representative of three independent experiments. **B.** SK-HEP-1 and SNU-387 cells were treated with either vehicle (DMSO) or KPT-330 (SK-HEP-1: 200nM; SNU-387: 300nM) for 72 hrs, stained and analyzed by flow cytometry. Histograms of Annexin V and Propidium iodide are shown. Numbers show the percentage of early apoptotic cells (Annexin V positive and propidium iodide negative; bottom right quadrant) and late apoptotic or necrotic cells (Annexin V positive and propidium iodide positive; top right quadrant). One representative of three independent experiments. **C.** SK-HEP-1, HepG2 and MHCC97H cells were treated with either vehicle (DMSO) or KPT-330 at the indicated concentrations for 72 hrs. Protein expression of BCL2, BAX and PUMA were determined by western blot analysis. β-actin

was used as the loading control. **D.** SK-HEP-1 and Huh7 cells were treated with either diluent control (DMSO) or increasing concentrations of KPT-330 for 48 hrs. Cells were stained with JC-1 and analyzed by flow cytometry. Upper box, green cells; lower box, red cells. The shift from red to green indicates mitochondrial membrane depolarization.

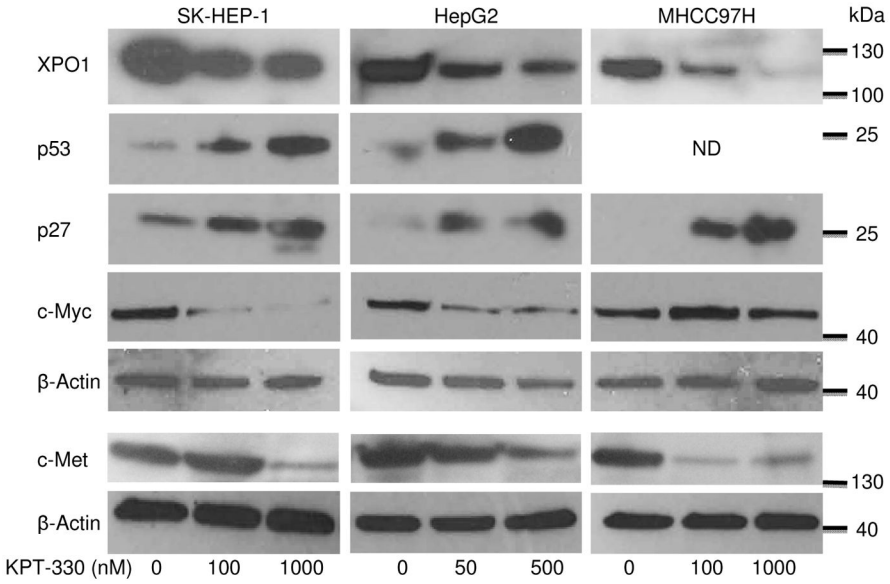

Figure 4.

KPT-330 increases expression of tumor suppressers and decreases expression of oncogenes. HCC cell lines were treated with either diluent (DMSO) or increasing concentrations of KPT-330 for 72 hrs. Lysates were analyzed by western blot for the indicated proteins. β-actin was used as the loading control. ND, not done.

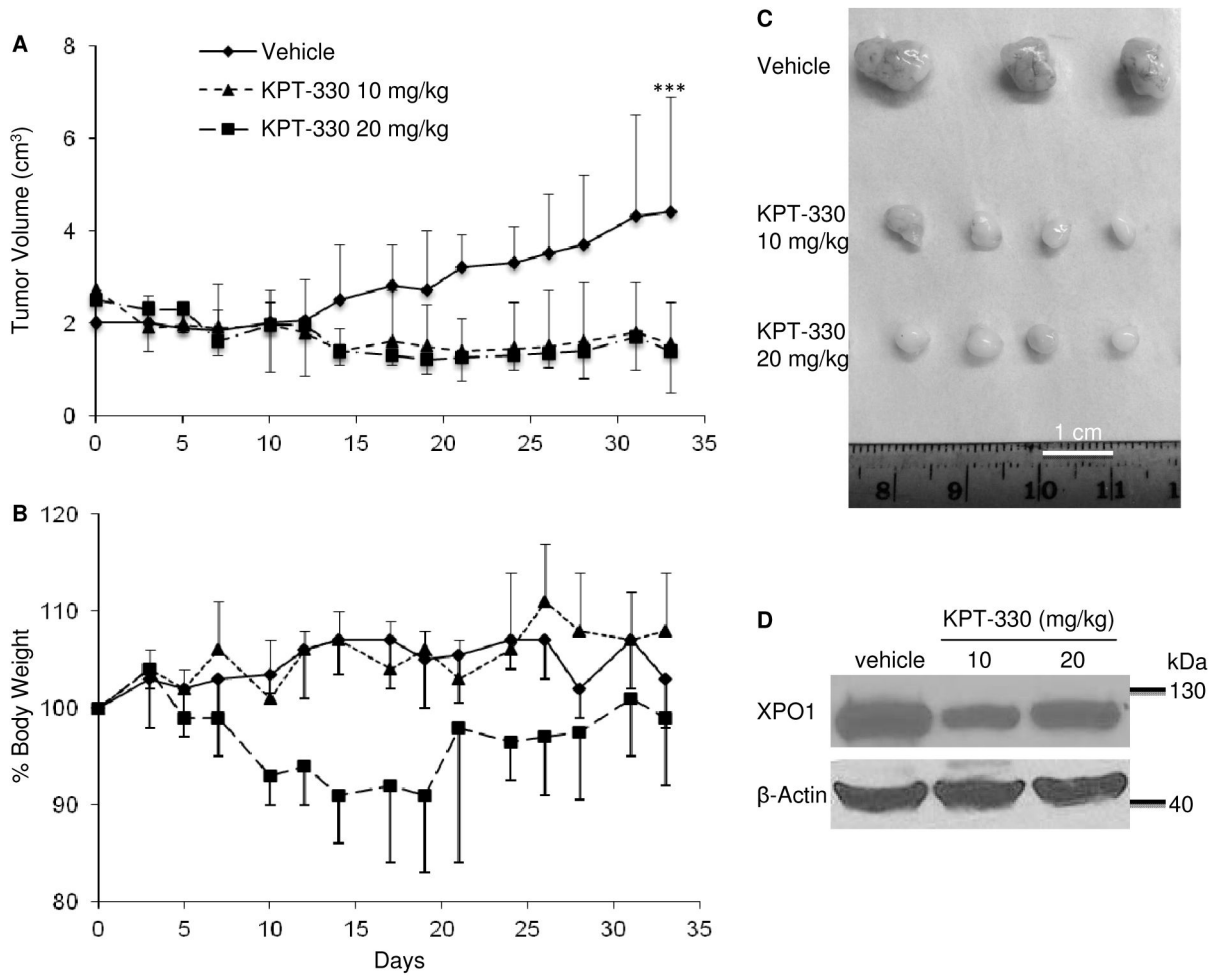

Figure 5. KPT-330 reduces tumor growth of HCC cells in a xenograft murine model

SK-HEP-1 cells (5×10^6) were subcutaneously injected into both flanks of nu/nu mice. Mice were treated with either vehicle control, low-dose (10 mg/kg) or high dose KPT-330 (20 mg/kg) three times a week for 28 days. **A.** Results represent the mean tumor volume (cm^3) of 10 tumors (five mice for each group). **B.** Evaluation of body weight after KPT-330 therapy. **C.** Representative tumors from each group. **D.** Western blot analysis of tumor lysates showing the *in vivo* effect of KPT-330 on XPO1 protein expression. ***, $P<0.001$.

Table 1

XPO1 expression in hepatocellular carcinoma

GEO Accession	Number of samples	Fold change	*p*-value	Ref
GSE6764	Normal liver: 10 HCC:35	1.8	2.65E-7	35
GSE14520	Normal liver: 220 HCC: 225	1.7	9.79E-54	36
GSE3500	Normal liver: 76 HCC: 104	1.5	1.55E-6	37
GSE14323	Normal liver: 19 HCC: 38	1.4	2.30E-5	38

Microarray data were obtained from Oncomine (www.oncomine.org); GEO, Gene Expression Omnibus; Fold change, XPO1 expression in HCC samples relative to normal liver samples.

Table 2

Sensitivity of hepatocellular carcinoma cell lines to KPT-SINE

Cell line	p53 status	IC_{50} at 72 h (nM)		
		LMB	KPT-276	KPT-330
SK-HEP-1	wild type	0.4	61.3	54.1
HepG2	wild type	0.6	160.4	45.1
Huh7	mutant	8.1	49.9	21.7
SNU-387	mutant	2.1	88.3	103.4
SNU-182	mutant	12.6	708.3	448.0
MHCC97H	mutant	ND	212.4	94.9

IC_{50}, concentration at which cell growth is inhibited by 50%; ND, not determine.