Selinexor demonstrates anti-tumor efficacy in paired patient-derived xenograft models and hydrogel-embedded histoculture drug sensitivity test of penile cancer

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Abstract

Background:

Penile cancer is a rare malignancy with a poor prognosis, even with various treatment options. Considering the little progress in the study of the pathogenesis and treatment of penile cancer because of the lack of models that mimic the biological properties of the tumor, we have developed a patient-derived xenograft (PDX) model and paired hydrogel-embedded histoculture drug sensitivity test (HDST) to screen for drugs that can inhibit tumors. The increased expression of XPO1, as a key nuclear export protein involved in the transport of various tumor suppressors and cell cycle regulatory proteins, is associated with the prognosis of a variety of tumors(MC et al., 2009). Selinexor is an inhibitor of XPO1, which can treat cancers such as multiple myeloma, gastric cancer, triple-negative breast cancer, and non-small cell carcinoma(A & TA, 2017) (P, I, M, & E, 2020). However, whether XPO1 inhibition has a role in penile cancer remains unknown. Therefore, this article used the PDX and HDST models to investigate whether the inhibition of XPO1 has an effect on penile cancer and its underlying mechanism.

Methods:

We used penile cancer tumor tissues to construct a PDX model of penile cancer and paired PDXE model and confirmed the consistency of PDX tumor tissues in source patients. Then, we assessed the ability of Selinexor to inhibit penile cancer tissues in vivo by using a PDX model and in vitro by HDST. We also examined the potential mechanism of XPO1 action on penile cancer by IHC and TUNEL. Finally, we assessed the safety of the drug treatment by H&E and biochemical blood analysis.

Results:

Result showed that the penile cancer PDX model and patient penile cancer tissues were clinically consistent in morphological characteristics and protein expression. In addition, Selinexor could inhibit tumor growth in PDX models and HDST. We found that P53, P21 expression was upregulated; Cyclin D1 expression was downregulated, and apoptosis of tumor cells was increased in the Selinexor-treated PDX model. Moreover, it had no significant effect on liver, kidney, and cardiac function.

Conclusion:

The PDX model of penile cancer was a powerful tool for penile cancer research and new drug development. It showed that Selinexor can effectively inhibit penile cancer in vitro and in vivo. In addition, XPO1 may affect P53, P21, and Cyclin D1 expression to regulate the growth and apoptosis of penile carcinoma.

Introduction

Penile cancer is a malignant tumor originating from the mucous membrane of the head, coronal sulcus, inner foreskin, and the skin of the penis(MM et al., 2016). It is the most common malignant tumor of the penis, accounting for over 90% of penile tumors. The most common pathological type of penile cancer is squamous cell carcinoma, which accounts for approximately 95% of penile cancers, whereas the other types are rare. It occurs in the inner foreskin plate and head of the penis, it and has a variety of histological subtypes that are pathologically similar to squamous cell carcinoma of other tissue origins(N & Z, 2012). For early-stage patients, surgical excision of the lesion is the primary and most effective treatment. Patients with advanced penile cancer and distant metastases, who were not suitable for surgical resections, should consider chemotherapy, and platinum and paclitaxel-based chemotherapy combined with inguinal lymph node dissection is the main treatment for advanced stages of this disease(Y et al., 2017). However, platinum-based chemotherapy regimens have low response rates and high toxicity. Therefore, new research is necessary to develop more effective and better tolerated systemic therapies. As penile cancer is rare, research on this type of cancer is insufficient and deficient, although literature has reported that cell line models of penile cancer have been established(M et al., 2014). However, these cell lines remain relatively few, and cell lines and cell line xenografts cannot reproduce the heterogeneity and microenvironment of the tumor; thus, the clinical translation rate of studies related to cell lines and cell line xenografts in penile cancer is poor(R et al., 2022).

A patient-derived xenograft (PDX) model is a tumor model generated by implanting fresh human tissue (tumor, enriched circulating tumor cells) into immunodeficient mice(J, Y, R, & X, 2020). The PDX model provides a complete tumor–host environment, preserving the histology, genomic features, and drug responsiveness of the original human patient tumor, which can be used as an “avatar” for studies on drug responsiveness studies(SY et al., 2016). The PDX model preserves the heterogeneity and microenvironment of the tumor, and the experimental results are up to 90% consistent with the clinical results(GJ, 2020). To date, a large number of PDX models of tumors have been constructed in humans, but the establishment of the PDX model of penile cancer has been rarely reported(J, HS, & S, 2018). Patient-derived Xenograft explants (PDXE) model is an in vitro culture and drug screening of tumor tissue from PDX tumor-bearing mice. The fragments

preserve the heterogeneity and microenvironment of the source patient's tumor (particularly the preservation of immune cells within the tumor). Thus, their drug screening results are highly compatible with the clinical results and are also used for tumor immune drug testing. The PDXE model is inexpensive, and it has a short testing cycle. The PDX model has the highest clinical agreement, and PDXE is characterized by high throughput. Therefore, the use of PDX tumor tissue and PDXE efficacy evaluation has short processing time, low cost, high throughput, and high in vivo and ex vivo result consistency. It is also in accordance with the reduction and substitution principle within the 3R principle of experimental animals. Our team has developed a new drug screening method based on histoculture, which is known as hydrogel-embedded histoculture drug sensitivity test (HDST). HDST drug screening using PDX tumor-bearing mouse tissue fragment is a kind of PDXE. HDST drug screening may improve the inconsistency of ex vivo and in vivo results, reduce the amounts of mice used, and increase the success rate of new drug development.

Selinexor is an inhibitor of the human nuclear export protein XPO1(JC et al., 2021), which is a major protein that mediates multiple nuclear outputs and plays a crucial role in maintaining cellular homeostasis. XPO1 is an export receptor responsible for the nuclear-cytoplasmic transport of hundreds of proteins and multiple RNA species(NG & Y, 2020).Studies have shown that XPO1 was overexpressed in myeloma, lymphoma, ovarian cancer, brain glioblastoma, osteosarcoma, pancreatic cancer, cervical cancer, gastric cancer, and other malignant tumors. XPO1 is frequently overexpressed and/or mutated in human cancers and functions as an oncogenic driver. Therefore, suppression of XPO1-mediated nuclear export presents a unique therapeutic strategy(GL et al., 2014). On July 3, 2019, Selinexor was approved by the US Food and Drug Administration for the treatment of relapsed refractory multiple myeloma(P et al., 2020). Selinexor has also been reported to treat other tumors, but whether it could treat penile cancer remains unknown(AL et al., 2021).

In this study, we established a penile cancer PDX model to explore the effect and mechanism of XPO1 on penile cancer by using the PDX models and HDST. In addition, the safety of Selinexor against penile cancer was tested. The results of this study could provide experimental basis for the clinical application of Selinexor.

Materials And Method

Patient and Tissue Samples

Patient's tissue samples were collected with the approval of the ethical committee of the Second Affiliated Hospital, Nanchang University. The recruited patient was required to sign an informed consent before sample collection. The patient was a 76-year-old male. A penile mass was found 1 month ago. CT showed an irregular soft tissue mass on the head of the penis (Fig. 1) with a right inguinal enlarged lymph node. The samples were pathologically diagnosed as penile cancer.

Materials

Balb/c nude mice aged 6–8 weeks (GemPharmatech Co., Ltd., Jiangsu, China) were used for PDX establishment and treatment. They were reared in an SPF environment. Selinexor (KTP-330) was purchased from BiochemPartner (China). Cisplatin was purchased from Haosen Pharmaceutical Co., Ltd. (Jiangsu, China). All animal studies were approved by the Institutional Animal Care and Use Committee of Nanchang Royo Biotech Co., Ltd. (RYE:2020071001).

Establishment of the PDX and PDXE penile cancer model

First, the tumor tissue was surgically removed from the patient. Fresh surgically resected penile cancer tissue was cut into small fragments of 2 mm × 2 mm × 2 mm and aseptically inoculated subcutaneously to the scapula of mice within < 24 h. The initially inoculated mice were numbered as P0. When the tissues developed tumors, the tumor size was measured using a vernier caliper, and the tumor volume (mm^3) was calculated by using the following formula: $V = a \times b^2 / 2$, where V represents tumor volume; a and b represents the longest and shortest tumor diameters, respectively. When the tumor volume increased to more than 1000 m^3, it was serially passaged in mice to P5 by using the same method, and P5 mice were used for subsequent experimental studies (Fig. 2).

During the acquisition of the PDXE model, penile cancer tissue originated from PDX mice was minced into blocks (2 mm × 2 mm × 2mm) and seeded in deep 96-well culture plates. Then, 150 µL of hydrogel was added to wrap the tissue block, which was used for subsequent drug test.

H&E staining

Tumor tissues from patients and PDX models were fixed in formalin, paraffin embedded, prepared into 4 µm dry slides, and stained with hematoxylin–eosin (H&E) by automatic slide stainer (DAKEWE Slide stainer DP360 Series).

Immunohistochemistry (IHC)

Immunohistochemical staining of p16 (Gene Tech), ck5/6 (Changdao), p63 (Gene Tech), ki67 (Changdao), CyclinD1 (Gene Tech), P21(Gene Tech), P53(Gene Tech), and XPO1(Proteintech) utilized a BenchMark XT Platform (Roche, Basel, Switzerland) as instructed.

Polymerase chain reaction (PCR)

RNA was extracted on ice from PDX tumor tissues. Primer primir5.0 design was used to design human- or mouse-derived genome-specific primers (Table 1). The extracted sample was amplified by PCR. Five microliters of DNA marker (DL2000) was added as a reference for the length of the amplified fragment. Electrophoresis was performed at 100 V for 15 min. When the indicator bromophenol moved to 2/3 of the gel, electrophoresis was terminated. The PCR product was observed in the DNA gel electrophoresis imager and photographed for analysis.

Table 1
DNA primers

Primers	Sequences	PCR products(bp)	Species
Mus-F1	CAGGTTGTCTCCTGCGACTT	571	Mouse genome
Mus-R1	CAGCTGGATGTCAGAGCCAA		
mus-F2	AAGGGCATCTTGGGCTACAC	549	Mouse genome
mus-R2	CCTGCTTCACCTCCCCATAC		
hs-F1	GGCTCTTAAAAAGTGCAGGGTC	327	Human genome
Hs-R1	ATGGTACATGACAAGGTGCGG		
hs-F2	TAACTGTCTGCTTCTCTGCTGTAGGC	772	Human genome
Hs-R2	GCTTCACCACCTTCTTGATGTCATCA		

PDXE culture with HDST

The PDXE model tissues were cultured with 700 μL of tissue culture medium containing 1.25, 2.5, 5, 10, and 20 μmol/L of Selinexor. After 3 days, the supernatant was replaced with a fresh medium containing an equal amount of drug (Fig. 2). Cell viability was detected by CCK8 assay after 7 days of treatment. All methods were performed in accordance with RoYo's instructions, and the kits were also obtained from ROYO.

PDX drug sensitivity test

The P6 generation PDX model was used for the experiment. Drug administration was initiated when the tumor volume reached 40–80 mm^3. Twenty-four mice were equally randomized into three groups: blank control group; cisplatin (CDDP) group, 5 mg/kg, intraperitoneal (IP) injection once a week; and Selinexor group, 20 mg/kg, intragastric administration (PO) two times a week. Mouse body weight and subcutaneous tumor size were measured two times a week. After 18-day drug administration, the animals were sacrificed. The tumors were excised and weighed. Each tumor tissue was cut into two halves. One half was fixed in formaldehyde, and the other half was stored at − 80 °C. Major organs, including the heart, liver, spleen, lung, and kidney, were removed and fixed in formaldehyde for histopathological assessment.

TUNEL assay

A TUNEL kit (Roche) was used to evaluate apoptosis. The formalin-fixed tissues were sliced to 4 μm, followed by xylene and graded ethanol treatment. The objective tissue was marked with a liquid blocker pen and then added with proteinase K working solution for antigen retrieval and permeabilized working solution for permeabilization. After incubation in buffer at room temperature, the reaction solution in the TUNEL kit was collected and covered. Then, the nucleus was counterstained with DAPI and finally mounted. Microscopic examination and image collection were performed using a fluorescence microscope.

Biochemical blood analysis of mice

Mice were euthanized by phenobarbital sodium, and blood was collected by cardiac puncture and then analyzed by microfluidic microarray in a Vet Chemistry Analyzer SMT-120VP (Chengdu Seamaty Technology Co., Ltd.), which is an automatic biochemical analyzer for animal diagnosis.

Statistical analysis

All calculations were performed by Prism 6. The results are expressed as the means ± SEM. Comparisons among multiple groups were made using two-way ANOVA. $P < 0.05$ was considered statistically significant difference.

Result

Tissues from the PDX model and patient with cancer were consistent in morphological characteristics and protein expression

We observed the morphological characteristics of the tumor tissue and PDX model by H&E staining. The tissue morphology of the PDX model is similar to that of the patient with caner, with less differentiated cells, nest-like arrangement of cancer cells, and evident heterogeneity (Fig. 3A). The PDX model tumor tissue maintains the morphological characteristics of the tumor from the patient. In addition, keratinized beads were present in the patient tissue but not in the PDX model, which indicates that the differentiation of tumor tissue from the PDX model is reduced. Genomic DNA was extracted from established PDX model tissues, amplified by PCR, and analyzed. The PDX tissue samples contained mouse and human target genes (Fig. 3B), indicating that the PDX model that we have established was derived from human tissue.

Then, p16, ck5/6, p63, and ki67 were selected as markers for IHC to demonstrate the consistency of protein expression. Human papillomavirus (HPV) is a risk factor for penile cancer. P16 may be an important alternative marker to confirm the diagnosis of precancerous and malignant lesions in HPV infection. Squamous cell carcinoma accounts for approximately 95% of all malignant diseases of the penis. CK5/6, a basal cytokeratin, is a marker for squamous cell carcinoma, and it demonstrates that the tumor in this experimental patient was a malignant tumor of squamous epithelial origin(Y et al., 2015). Some studies have found that P63 is closely related to the development of squamous carcinoma(G, 2011). Thus, the detection of P63 protein may have early diagnostic significance for malignant tumors of squamous epithelial origin. Ki67 antigen is an intranuclear division and proliferation-associated protein. It is an important indicator of tumor activity, and high Ki67 expression indicates strong proliferative activity and high invasiveness of tumor cells. The result showed that the characteristics of the PDX model that we established were consistent with the histological and immunohistochemical characteristics of the primary tumor (Fig. 3C).

Selinexor effectively inhibited the activity of penile cancer cells in the HDST model

In testing Selinexor as an inhibitor of penile cancer in vitro, we tested the cell activity of Selinexor against penile carcinoma using the HDST model. We treated tumor tissue with 1.25, 2.5, 5, 10, and 20 μmol/L, respectively, and the results showed that Selinexor at approximately 5 μmol/L had a more significant inhibitory effect on penile cancer, which was concentration dependent within a certain range (Fig. 4).

Selinexor effectively inhibited penile cancer growth in the PDX model

Patient tumors were implanted in nude mice to investigate the inhibitory effect of Selinexor on tumor growth in vivo. A total of 18 model mice were randomly divided into three groups: the blank control group, the cisplatin group (intraperitoneal injection of 5 mg/kg once a week), and Selinexor group (gastric lavage 20 mg/kg two times a week). Selinexor and cisplatin were administered in accordance with the aforementioned regimens. After 21 days, effective inhibitory effects on tumor growth were observed in the cisplatin and Selinexor groups, particularly in the Selinexor group, where Selinexor had the most significant inhibitory effect (Figs. 5A–D). In Selinexor and cisplatin treatment groups, tissue fibrosis and inflammatory cell infiltration were evident (Fig. 5E).

Selinexor inhibited penile cancer XPO1 and Cyclin D1 expression, as well as promoted P53 and P21 protein expression and induced cell apoptosis

Selinexor is the inhibitor of XPO1. Cyclin D1 is a cell cycle–related protein, which primarily promotes cell proliferation. Cyclin D1 has been recognized as a proto-oncogene, whose overexpression can lead to uncontrolled cell proliferation and malignancy(R, GN, D, A, & A, 2014).P53 is a tumor suppressor protein, and inhibition of XPO1 by Selinexor promotes the accumulation of p53 in the nucleus, allowing p53 to fully exploit its tumor-suppressive function(MJ, NC, & J, 2017). P21 is a cytokine-dependent kinase inhibitor downstream of the P53 gene, which is associated with tumor differentiation, tumor differentiation, infiltration depth, proliferation, and metastasis, indicating its prognostic value(AG, OA, & WM, 2017). In this experiment, the XPO1 expression level was downregulated, whereas P53 and P21 expression levels were upregulated, indicating that Selinexor exerted its oncogenic effect by inhibiting nuclear translocation in penile cancer, leading to the

accumulation of P53 and P21 in the nucleus, which is consistent with the previous findings (VV et al., 2018) (AG et al., 2017)[21][21]. TUNEL staining can detect the breakage of nuclear DNA during apoptosis(K, S, M, & C, 2012).

In investigating the mechanism of Selinexor in inhibiting tumor growth, we used IHC to observe the difference in the expression of XPO1, P53, P21, CyclinD1 in the control and Selinexor groups, and TUNEL to determine the number of apoptosis in cancer cells. The expression level of XPO1 and Cyclin D1 was downregulated, whereas that of P53 and P21 was upregulated (Fig. 6A). The TUNEL experiment showed that apoptosis was significantly increased in the Selinexor group (Fig. 6B).

Selinexor treatment was safety for mice

In investigating the safety of Selinexor treatment in mice, we observed the heart, liver, spleen, lungs, and kidneys by H&E staining. It showed that the heart, liver, spleen, lungs, and kidneys did not show major organ-related toxicity in all treatment groups (Fig. 7). The result of biochemical *blood analysis of mice showed that the Selinexor treatment had no organ-related toxicity (*Table 2*).*

Table 2
Biochemical blood analysis of mice after Selinexor treatment

Group	*n*	TP(g/L)	AST(U/L)	ALT(U/L)	ALP(U/L)	LDH(U/L)	CK(U/L)	Crea (umol/L)	UREA (umol/L)	U/C	GLU (mmol/L)
Control	8	59.89 ± 5.23	240.13 ± 160.56	60.25 ± 25.53	109.88 ± 33.02	967 ± 243.67	1907.25 ± 1457.32	13.41 ± 9.53	8.64 ± 0.83	434.42 ± 129.05	6.39 ± 2.25
Selinexor	7	58.73 ± 4.79	189.71 ± 96.58	84.14 ± 58.61	154.86 ± 34.03	982.29 ± 229.33	1178.71 ± 1254.65	16.64 ± 8.74	10.73 ± 1.49	509.73 ± 81.03	6.33 ± 1.46

TP, total protein; AST, corn transaminase; ALT, glutamate transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase; Crea, creatinine; UREA, urea; GLU, blood glucose

Discussion

Penile cancer can constitute up to 10% of male malignancies in some African, Asian, and South American areas (A et al., 2021). We successfully constructed a PDX model of penile cancer. After identification, PDX and the source of the patient's swelling tumor morphology and biological characteristics are consistent. The PDX model has many advantages, which have been widely cited in many types of tumor research(M et al., 2014). However only one European team constructed a penile cancer sample bank consisting of 11 penile cancer PDX models[25]. Our PDX model was derived from a Chinese Han male, which was cisplatin sensitive and P16 positive (HPV infection). Infection with HPV was a risk factor associated with penile cancer(KL & DJ, 2016). A recent large meta-analysis reports a pooled HPV prevalence of 50.8% in penile cancer(TB et al., 2019). Therefore, our PDX model of penile cancer will be an important research tool for penile cancer research.

The PDX model is a common drug screening model in clinic, but it is expensive and time consuming. Using a PDX model of tumor tissues for HDST drug screening can shorten the experimental period, improve the drug screening flux, and conform to the 3R principle of experimental animals. PDXE has been reported in some literature(C. A et al., 2020). HDST screening is a PDXE screening method independently developed by our team. Compared with other PDXE screening methods, HDST screening has better simulation of the tumor growth environment (mechanical pharmacology), convenient operation, and high consistency between screening results and clinical practice.

Treatment of penile cancer is unsatisfactory; thus, new treatments are needed. We used the HDST model of penile cancer to screen a variety of marketed anticancer-targeted drugs. The XPO1 inhibitor Selinexor was found to have a significant effect on penile carcinoma tumor tissue, which was the same as the patient-derived PDX model. Platinum is a classic drug for penile cancer(J, J, & PE, 2022). However, the patient of our penile cancer PDX-derived gave up treatment and did not take it. Therefore, the PDX model has a certain sensitivity to cisplatin treatment, which is consistent with the literature. Our in vivo results show that Selinexor is less tumor bearing than cisplatin. In addition, our results are consistent with those reported in the literature, that is, XPO1 plays an important role in regulating tumor cell proliferation and apoptosis. The positive expression of XPO1 in the PDX model and in clinical samples of patients with penile cancer indicates that the expression of XPO1 is closely related to the occurrence and development of penile cancer. Although the mechanisms underlying the selective antitumor effects of Selinexor remain unclear, our results indicate that Selinexor inhibits the nuclear export of p53 by blocking XPO1 and reducing the degradation and functional inactivation of p53 as reported in the literature. p21 induces cell cycle arrest in response to DNA damage. Moreover, we hypothesize that the upregulation of p21, a transcriptional p53 target, promotes the restoration/reactivation of p53 tumor suppressor function after Selinexor treatment. On the contrary, XPO1 may affect the depolymerization and function of Cyclin D1, which promote cell proliferation and differentiation, thereby leading to increased apoptosis.

Our in vivo results show that Selinexor has a better inhibitory effect than cisplatin on penile cancer in tumor-bearing mice. Selinexor is safe and accessible, and it is available on the market. It may play an important role in the clinical treatment of penile cancer in the future. Our experimental results show that the HDST screening results were consistent with the PDX results, and the paired HDST/PDX model has important application value in anticancer drug screening.

Conclusion

In this study, we successfully established the PDX model of penile cancer and proved its consistency with clinical patients, thereby providing a powerful tool for the treatment research and new drug development of penile cancer. In addition, we used PDXE drug screening to find a new drug Selinexor for the treatment of penile cancer and proved that Selinexor has an inhibitory effect on the growth of the PDX model of penile cancer, providing a preliminary research basis for clinical patients.

Declarations

Data availability statement

All datasets generated for this study are included in the article/Supplementary Material.

Ethics statement

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanchang Royo Biotech Co., Ltd. (Permit RYE2022040301) The patient had signed the informed consent form, and the sample collection was approved by the medical ethics committee of the Second Affiliated Hospital of Nanchang University. (Permit No.2021012). Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Consent for publication

Informed consent was obtained from the patient and his relatives.

Author contributions

Xiongbin Lu and Yuanqiao He conceived of the study. Yuanqiao He, Mei Jiaqi, Hua Hao, Fanrong Liu and Yun Yi performed experiments and analyzed date. Xiongbin Lu and Yuanqiao He wrote the manuscript.

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Figures

Fig. 1

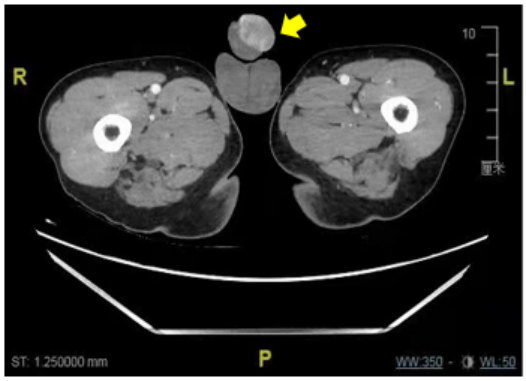

Figure 1

CT image of patient's tumor tissue; the tumor tissue is indicated by the yellow arrow

Fig. 2

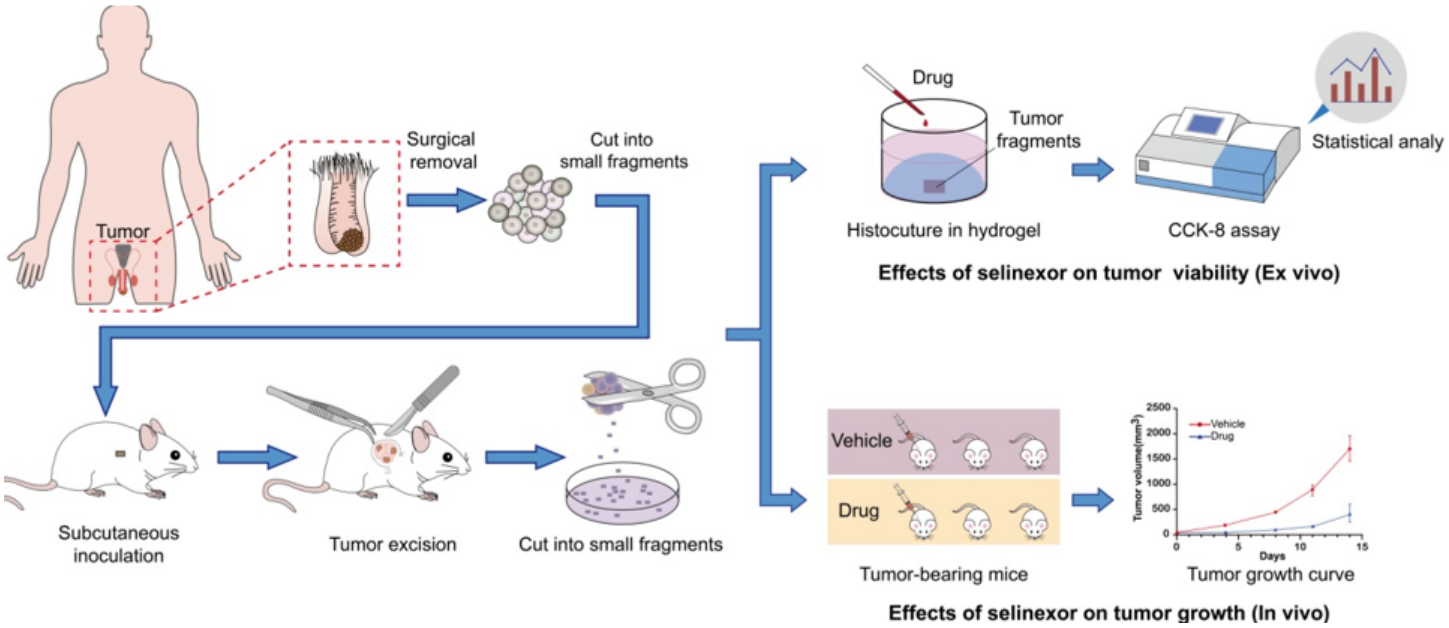

Figure 2

Basic flow of the PDX model and HDST model drug screening

Fig. 3

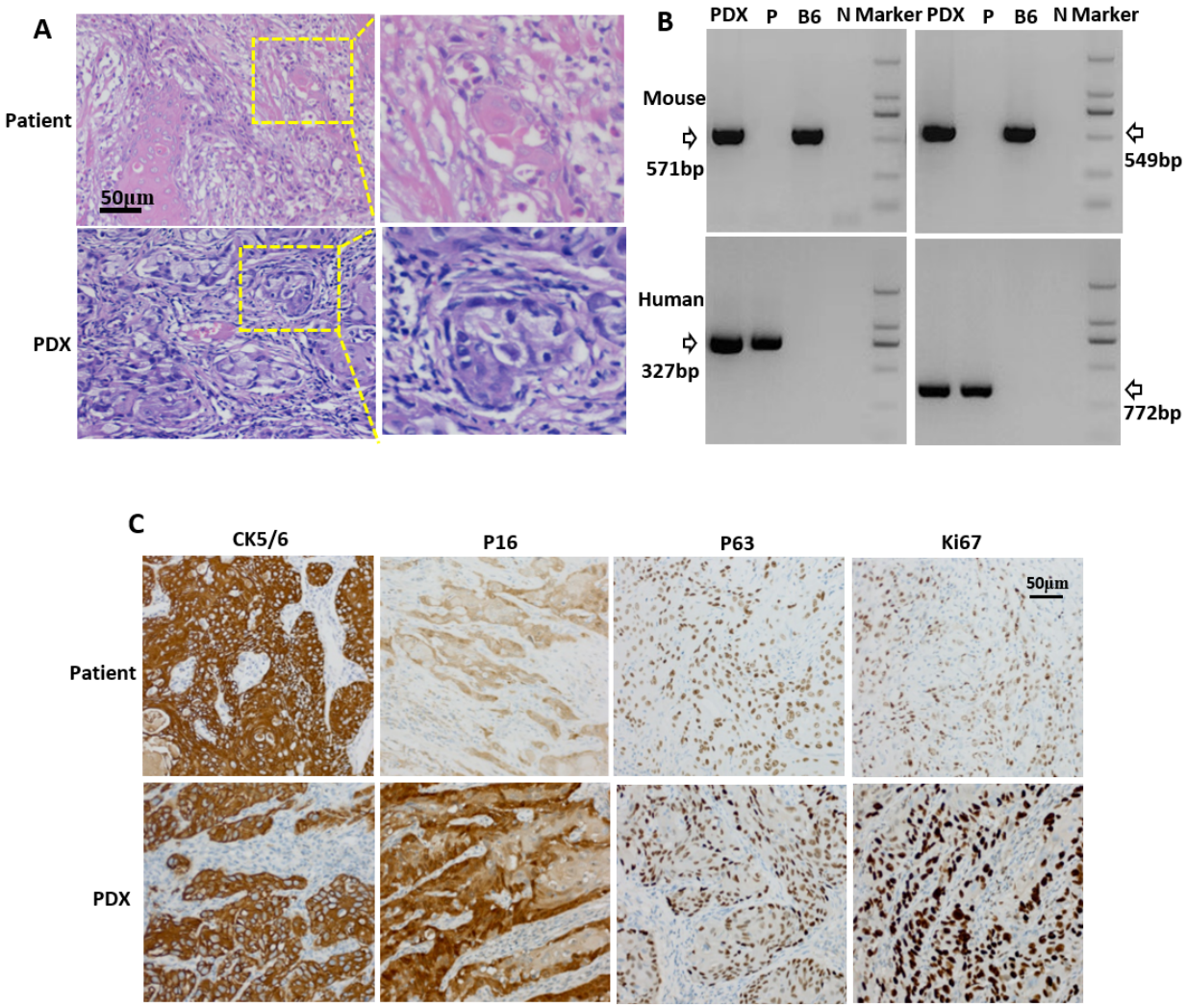

Figure 3

PDX model and patient cancer tissues were consistent in morphological characteristics and protein expression. *(A) Morphological characteristics were consistent in tumor tissue of patients and PDX model tissue. (B) PDX tissue samples contained mouse and human target genes: PDX stands for PDX model tissue; P stands for patient tissue; B6 indicates a positive control of mouse origin, and N indicates a negative control. (C) CK5/6, P16, P63, and Ki67 expression levels were consistent in the PDX model and patient cancer tissues.*

Fig. 4

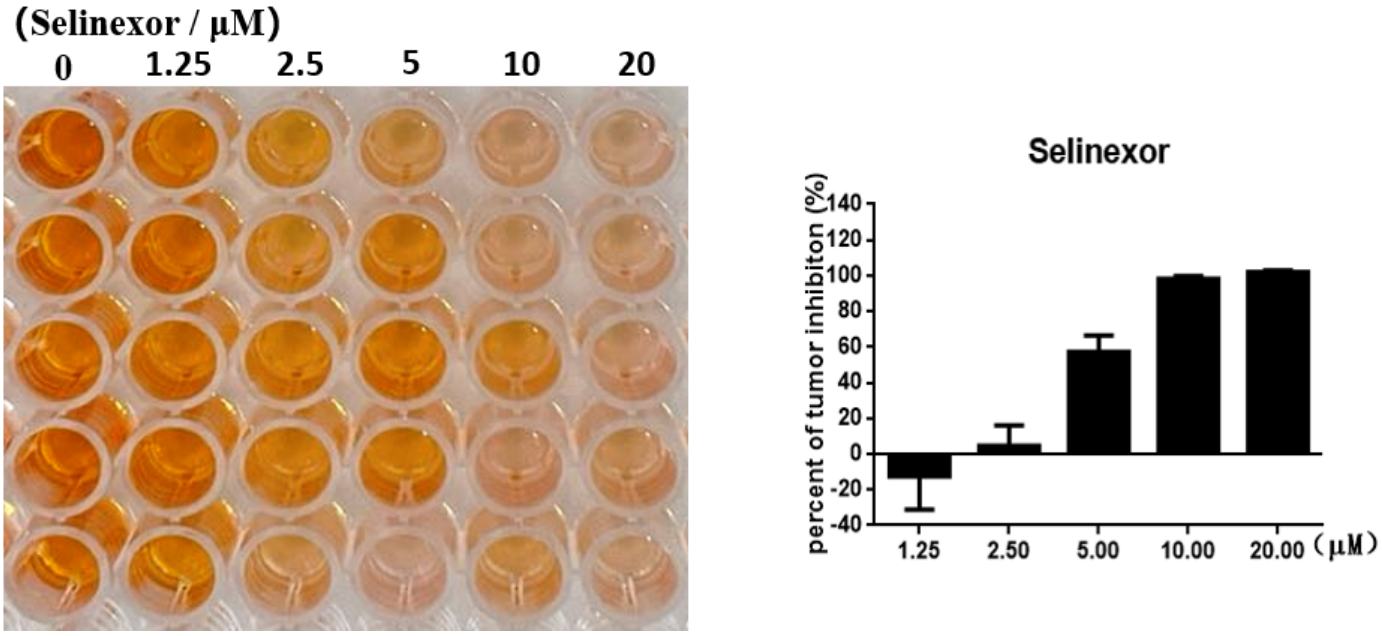

Figure 4

Selinexor inhibited the activity of penile cancer cells in vitro. *(A) HDST cell viability with Selinexor treatment was assayed by CCK8. (B) Statistical data showed that Selinexor inhibited the activity of penile cancer cells at 5, 10, and 20 μM concentrations (tumor growth inhibition rate greater than 40% was considered effective).*

Fig. 5

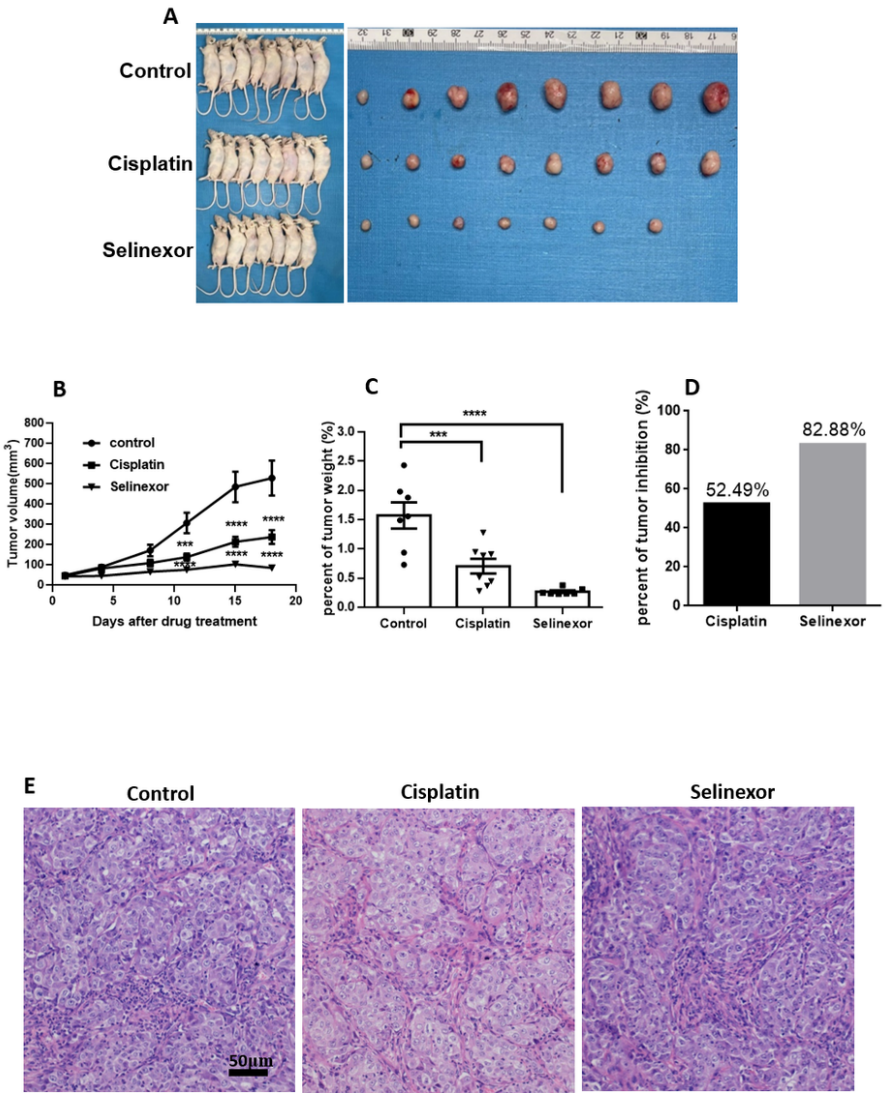

Figure 5

Selinexor effectively inhibited penile cancer in vivo. *(A) The PDX model showed that Selinexor inhibited penile cancer. (B–D) Statistical data showed that Selinexor inhibited the penile cancer volume, rate of tumor weight, and rate of tumor inhibition, ***p<0.001; **** p<0.0001. Tumor growth inhibition rate greater than 40% was considered effective. (E) Morphological characteristics were consistent with cisplatin and Selinexor treatment.*

Fig. 6

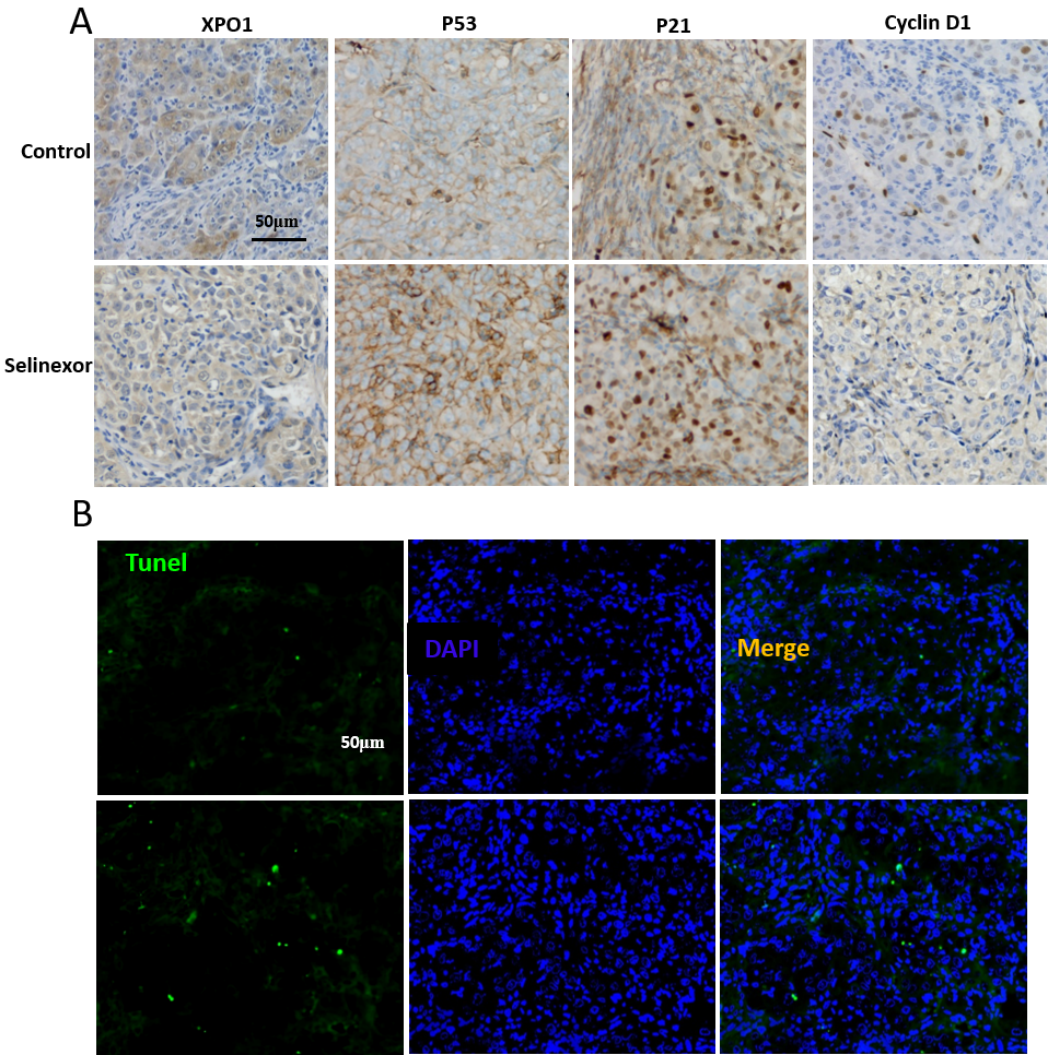

Figure 6

Selinexor affected the expression of XPO1, P53, P21, Cyclin D1, and cell apoptosis. *(A) XPO1 and Cyclin D1 expressed downregulation, and P53 and P21 expressed upregulation after Selinexor treatment. (B) Selinexor treatment induced increased apoptosis by TUNEL assay.*

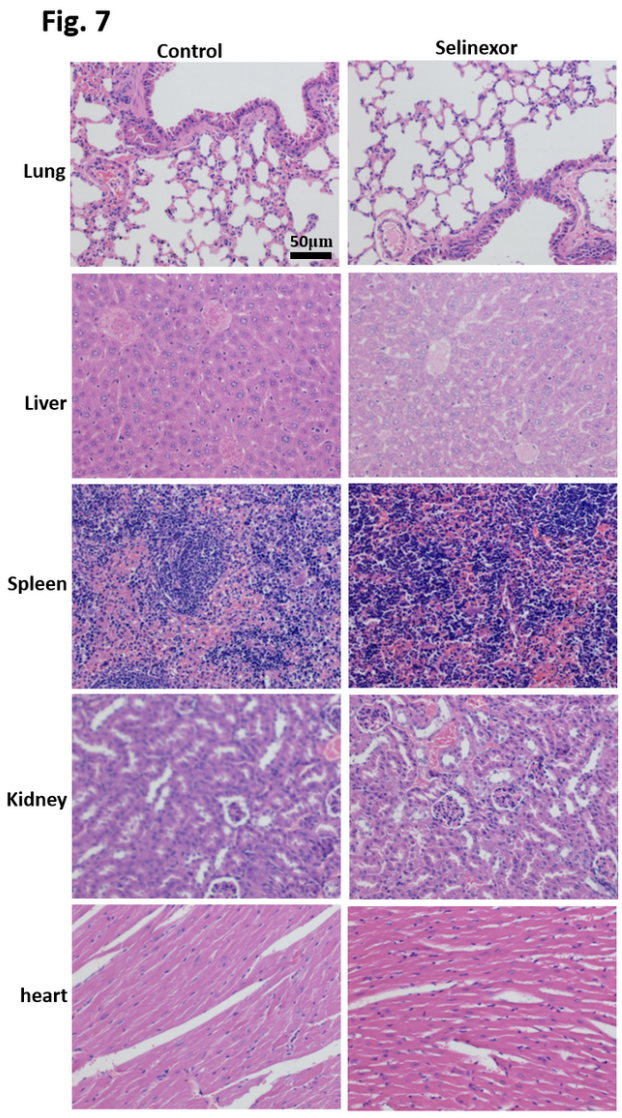

Figure 7

Heart, liver, spleen, lungs, and kidneys did not show major organ-related toxicity after Selinexor treatment.