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Targeting PP6C as an Inhibitor of PP2A for Cancer Therapy

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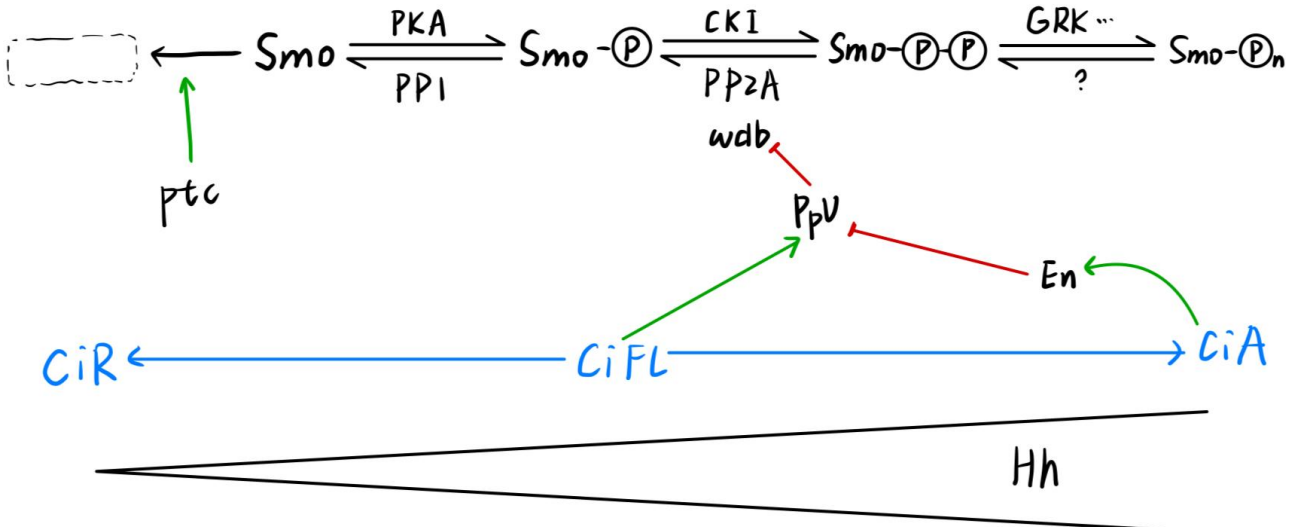
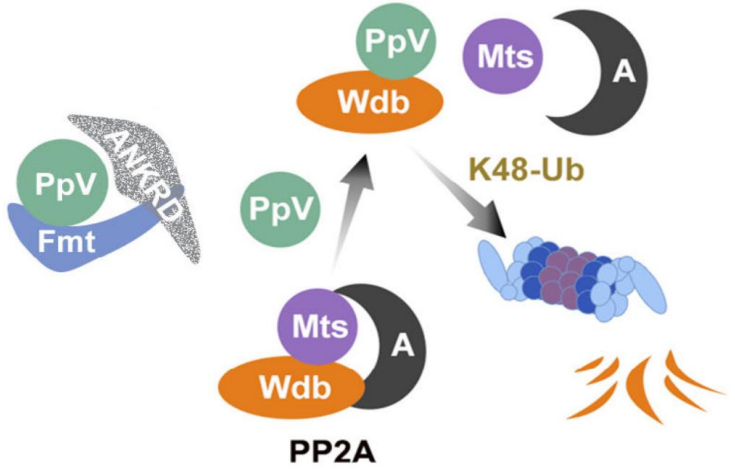
01

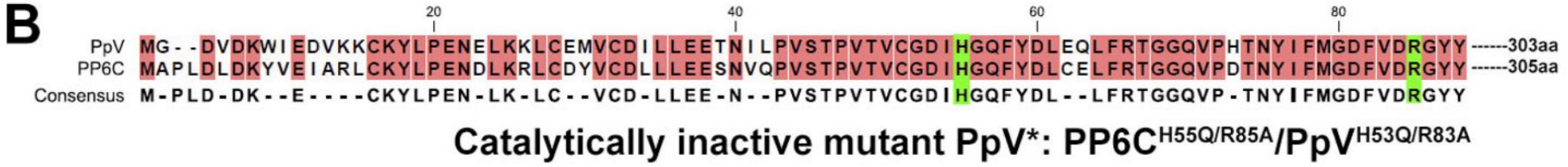
Background

Background

In Drosophila, PPV, the catalytic subunit of PP6, was found to down-regulate PP2A by competitively binding to wdb, the regulatory subunit of PP2A





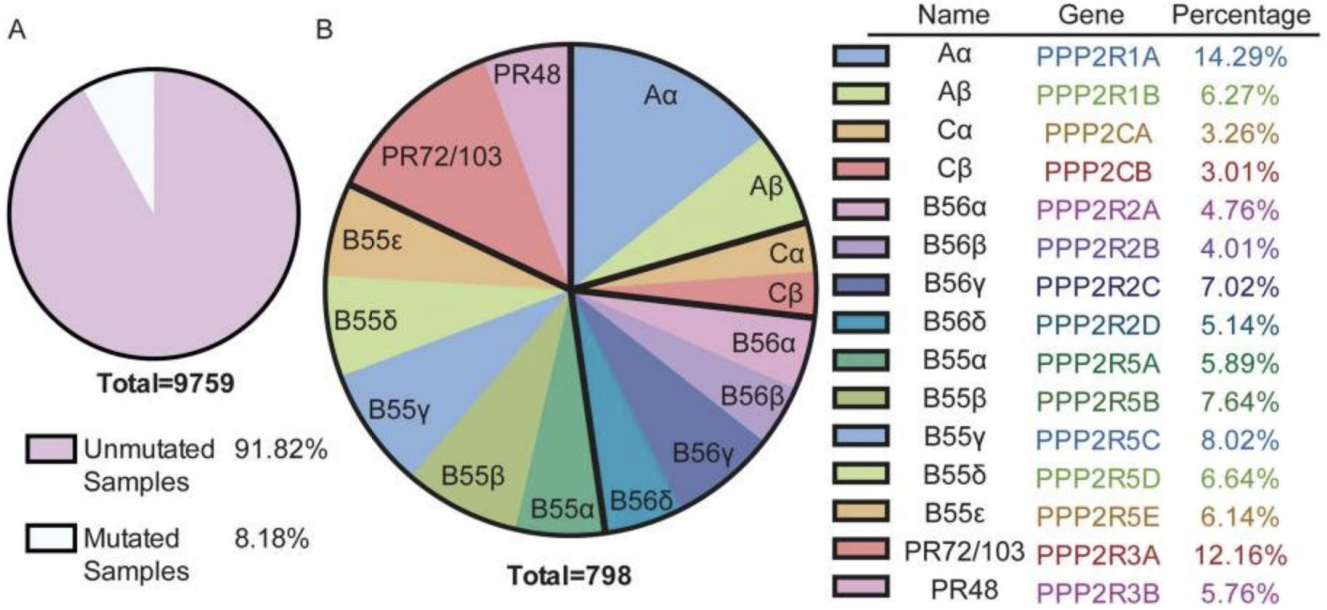


Catalytically inactive mutant PpV*: PP6C$^{H55Q/R85A}$/PpV$^{H53Q/R83A}$

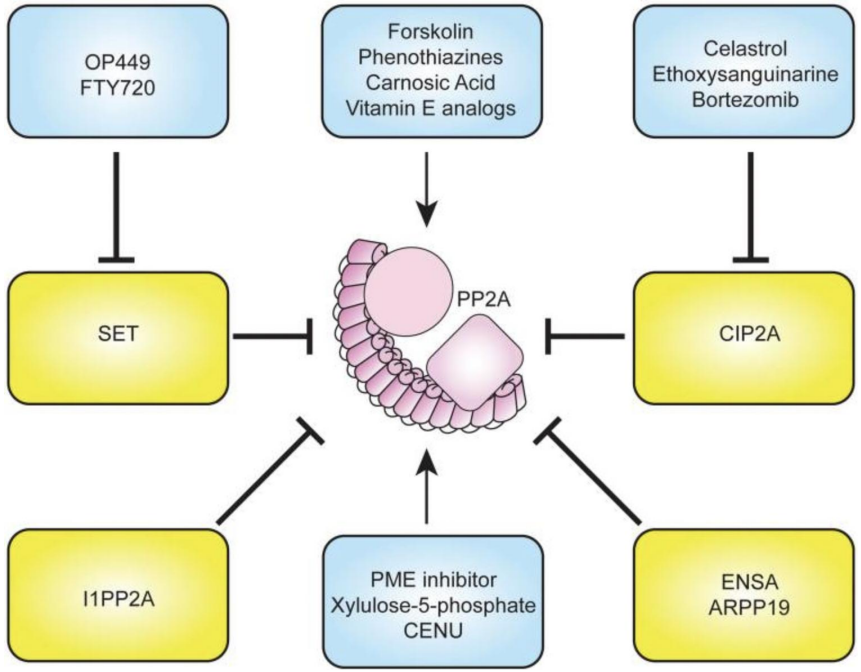
Liu et.al, 2020

Background

- **PP2A is a tumor suppressor gene, and its inactivation can lead to tumor disease**
- **PP2A has many endogenous inhibitors，which are the targets of many anti-cancer drugs**



Name	Gene	Percentage
Aα	PPP2R1A	14.29%
Aβ	PPP2R1B	6.27%
Cα	PPP2CA	3.26%
Cβ	PPP2CB	3.01%
B56α	PPP2R2A	4.76%
B56β	PPP2R2B	4.01%
B56γ	PPP2R2C	7.02%
B56δ	PPP2R2D	5.14%
B55α	PPP2R5A	5.89%
B55β	PPP2R5B	7.64%
B55γ	PPP2R5C	8.02%
B55δ	PPP2R5D	6.64%
B55ε	PPP2R5E	6.14%
PR72/103	PPP2R3A	12.16%
PR48	PPP2R3B	5.76%



Sangodkar,2016

Background

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- **Can the inhibitory relationship model of PP6C on PP2A be introduced into mice as endogenous inhibitors of PP2A for screening anticancer drugs?**

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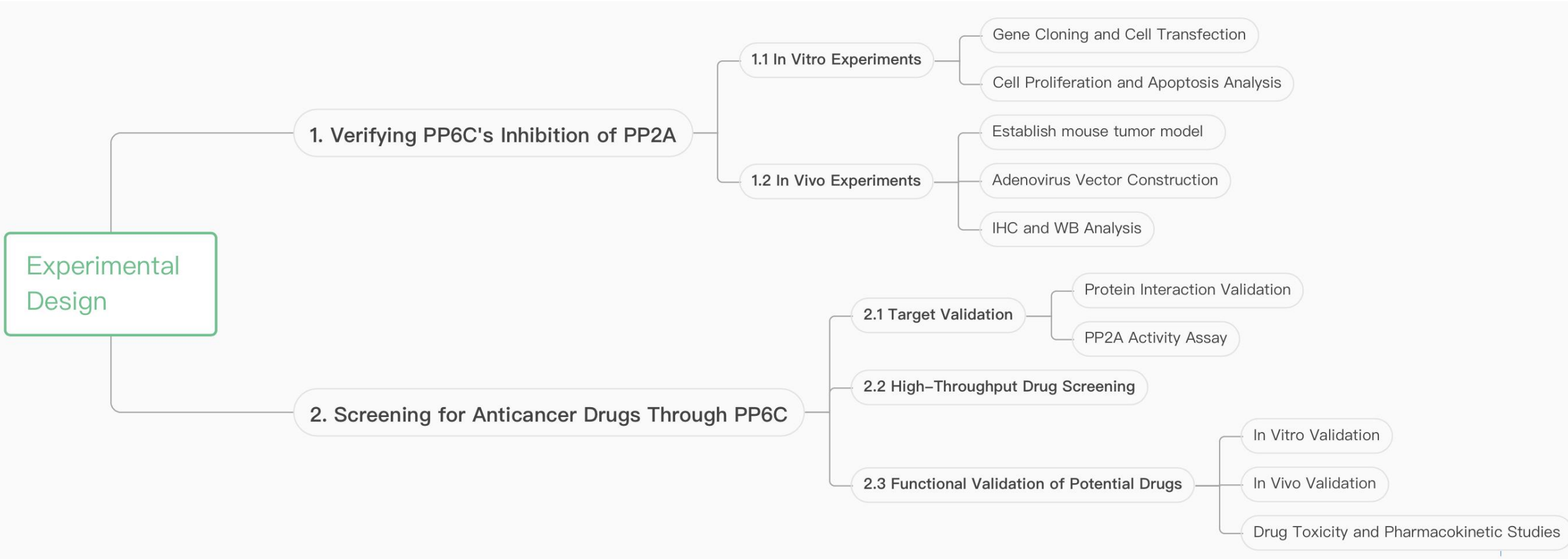
02

Designs and Methods

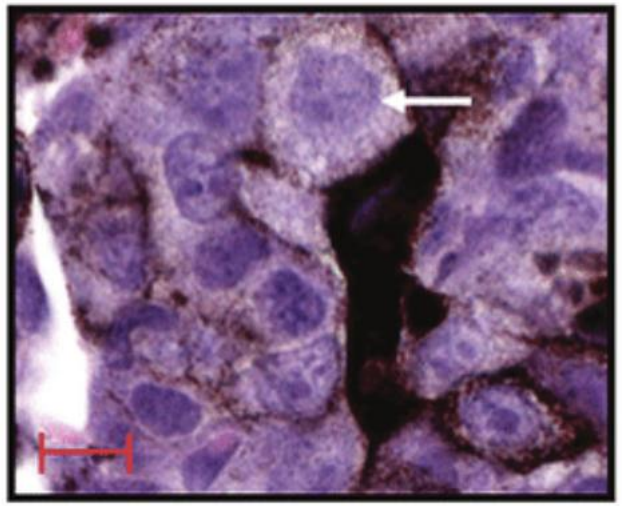
Brief


Experimental Design
1. Verifying PP6C's Inhibition of PP2A
1.1 In Vitro Experiments
Gene Cloning and Cell Transfection
Cell Proliferation and Apoptosis Analysis
1.2 In Vivo Experiments
Establish mouse tumor model
Adenovirus Vector Construction
IHC and WB Analysis
2. Screening for Anticancer Drugs Through PP6C
2.1 Target Validation
Protein Interaction Validation
PP2A Activity Assay
2.2 High-Throughput Drug Screening
2.3 Functional Validation of Potential Drugs
In Vitro Validation
In Vivo Validation
Drug Toxicity and Pharmacokinetic Studies

1.1 In Vitro Experiments

1. Cell Line Selection and Culture

- Select mouse embryonic fibroblasts (MEF) or mouse tumor cell lines (eg, B16 melanoma cells).
- **Culture cells** in DMEM medium with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO_2.



2. Gene Cloning and Plasmid Construction

- **PCR** Amplification: Design specific primers to amplify the PP6C gene.
- Plasmid Construction: **Clone the PCR product** into the **pGKTK vector** using restriction enzyme digestion and ligation.

3. Cell Transfection

- Transfect PP6C expression plasmid into mouse cells. Transfect control group with *empty vector plasmid.*

1.1 In Vitro Experiments

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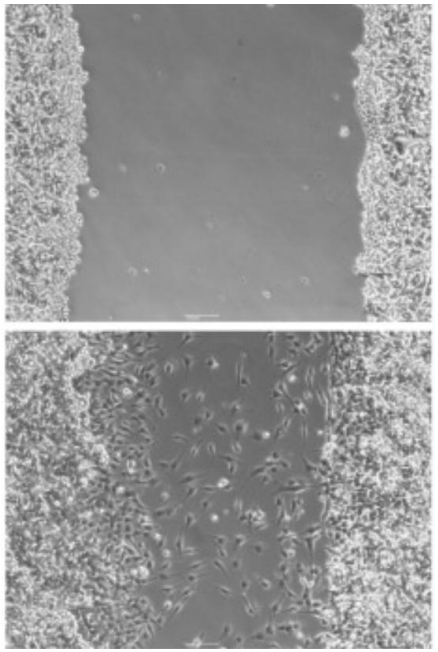
4. Protein Extraction and Western Blot

- Collect cells 48 hours post-transfection, lyse with RIPA buffer, and collect supernatant after centrifugation.
- Quantify protein, load equal amounts for **SDS-PAGE,** transfer to membrane, and incubate with antibodies. Detect using **ECL**.

RIPA buffer
Tris-HCl(pH 7.4)
150mM NaCl
1% NP-40
0.1% SDS

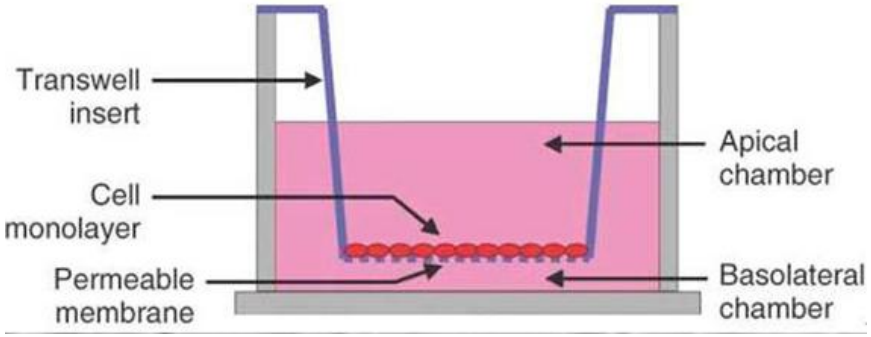
5. Cell Apoptosis and Migration Analysis

- CCK-8 Assay: Seed cells in a 96-well plate, add CCK-8 reagent, and measure absorbance at 450 nm.
- Migration Analysis：**Cell scratch assay** and **Transwell assay**



Expected result

- PP6C transfection group had **fewer** apoptotic cells than control group
- The cell migration rate of PP6C transfection group was **faster** than that of control group



1.2 In Vivo Experiments

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1. Mouse Model Selection

- Choose C57BL/6 mice, and subcutaneously inoculate them with B16 melanoma cells.

2. Adenovirus Vector Construction and Production

- **Adenovirus Vector** Construction: Clone PP6C gene into an adenovirus vector.
- Production and Purification: Amplify the adenovirus in HEK293T cells and purify viral particles by ultracentrifugation.

3. Adenovirus Injection in Micea

- Inject recombinant adenovirus Ad-PP6C via the tail vein when tumor volume reaches about 100 mm^3. Control group receives *Ad-GFP*.

1.2 In Vivo Experiments

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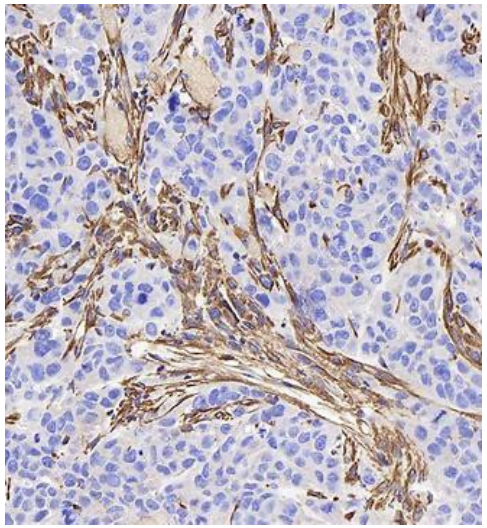
4. In Vivo Observation and Analysis

- Regularly measure and record tumor volume, and plot tumor growth curves.
- At the end of the experiment, euthanize mice, and collect tumor tissues and major organs for histological analysis.

5. Immunohistochemistry and Western Blot Analysis

- **IHC:** Stain tumor tissue sections with anti-PP2A antibody.
- **Western Blot:** Extract total protein from tumor tissues and detect changes in PP2A and its downstream signaling molecules.



Expected result

- IHC staining was **positive** in PP6C transfection group
- In the PP6C transfection group, the expression of PP2A **decreased**, the expression of downstream tumor suppressor gene decreased, and the expression of protumor gene increased

2.1 Target Validation

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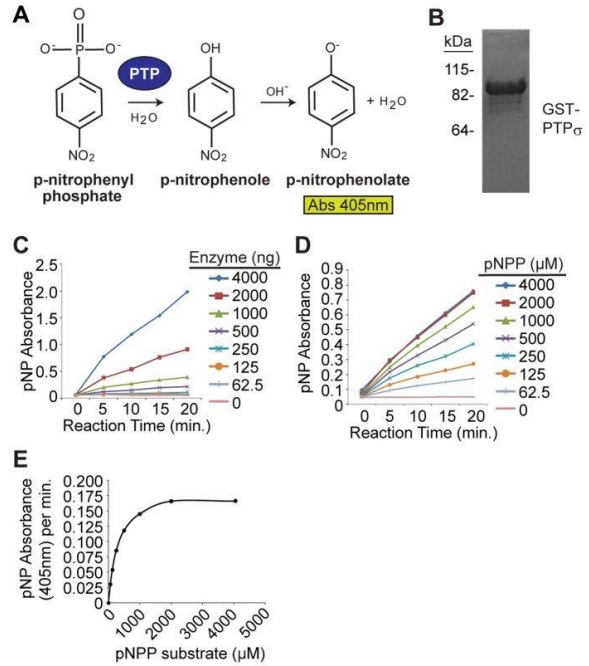
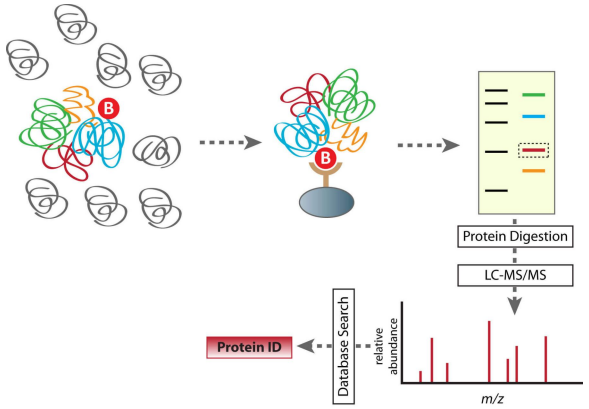
1. Protein Interaction Validation

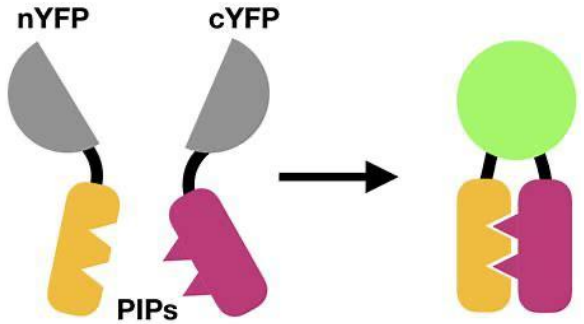
- Co-Immunoprecipitation **(Co-IP)**: Transfect PP6C-Flag plasmid, use anti-Flag antibody for immunoprecipitation, and detect PP2A regulatory subunits binding.
- Bimolecular Fluorescence Complementation **(BiFC)**: Construct BiFC reporter vectors for PP6C and PP2A regulatory subunits, and observe intracellular interactions.

2. PP2A Activity Assay

- Phosphatase Activity Assay: Extract protein from cells or tumor tissues, and measure PP2A activity using specific substrate (e.g., pNPP).







Expected result

- Cell interaction experiments showed positive results
- PP2A phosphatase activity decreased in PP6C transfection group

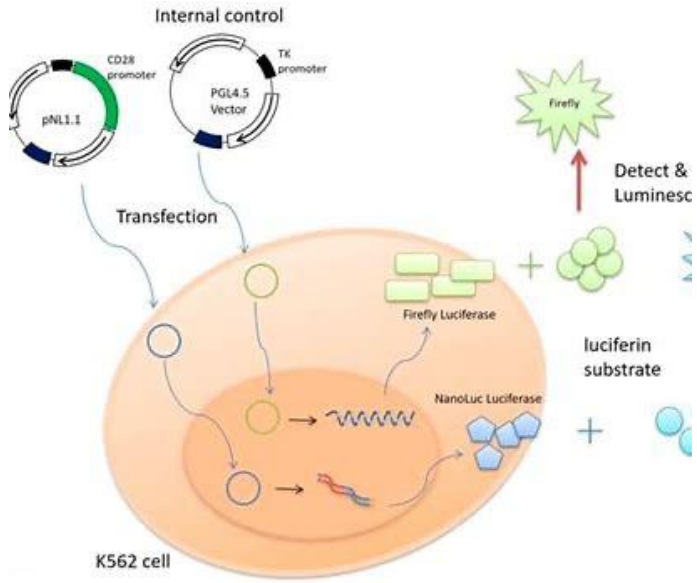
2.2 High-Throughput Drug Screening

1. Cell Model Construction

- Establish mouse cell lines or tumor cell lines stably expressing PP6C.
- Construct **lentiviral vector** containing PP6C, infect cells, and screen for stable clones.

2. Establishing High-Throughput Screening Platform

- Reporter System: Construct fluorescence or luciferase reporter system based on PP2A activity or its downstream signaling pathways.
- High-Throughput Screening (HTS): Seed cells in 96- or 384-well plates, treat with compound library, and detect changes in PP2A activity using the reporter system.



Expected result

- Compounds that meet the requirements emit significant fluorescence

2.3 Functional Validation of Drugs

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1. In Vitro Validation

- Cell Proliferation and Apoptosis Analysis: Use CCK-8, MTT, or migration analysis to assess the effects of compounds on the proliferation and apoptosis of PP6C stable cell lines.
- Western Blot: Detect the expression and phosphorylation levels of PP2A and its downstream signaling molecules after compound treatment.

2. In Vivo Validation

- Mouse Model Establishment: Treat tumor-bearing mice with the compounds, regularly measure tumor volume, and monitor overall health.
- Efficacy Evaluation: At the end of the experiment, collect tumor tissues and major organs for histological analysis.
- IHC and Western Blot: Detect changes in the expression and activity of PP2A and its downstream signaling molecules in tumor tissues.

2.3 Functional Validation of Drugs

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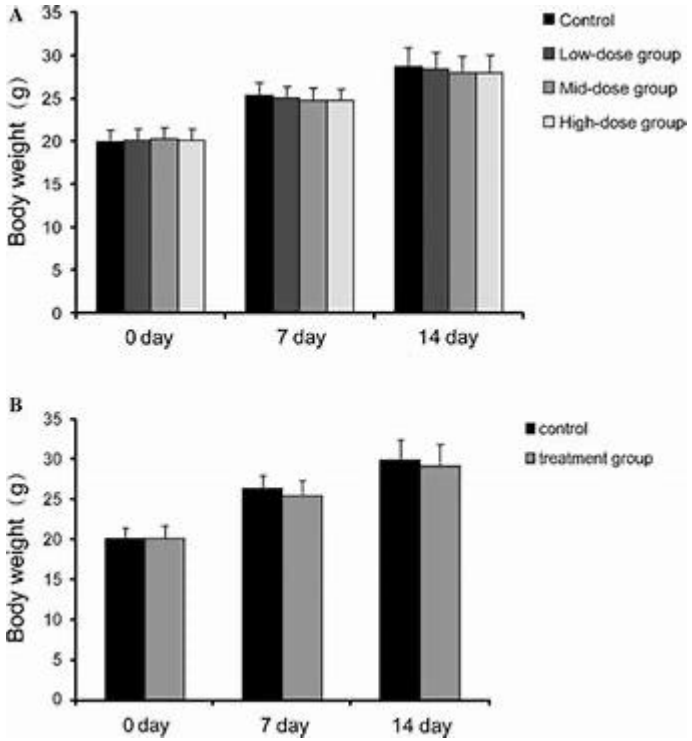
3. Drug Toxicity and Pharmacokinetic Studies

- **Acute Toxicity Test**: Test different doses of compounds, observe for 14 days, and record mortality and toxicity reactions.
- Maximum Tolerated Dose (MTD): Determine the highest dose that does not cause severe toxicity.
- **Long-Term Toxicity Test**: Conduct long-term toxicity tests based on MTD, monitor physiological indicators and pathological analysis of major organs.
- **Pharmacokinetic Analysis**: Use LC-MS/MS to detect the absorption, distribution, metabolism, and excretion (ADME) of compounds in mice.



Drugs with low acute and long-term toxicity are possible anticancer drugs

Clinical tests……

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Discussion

Discussion

- **Selection of transfection vector**

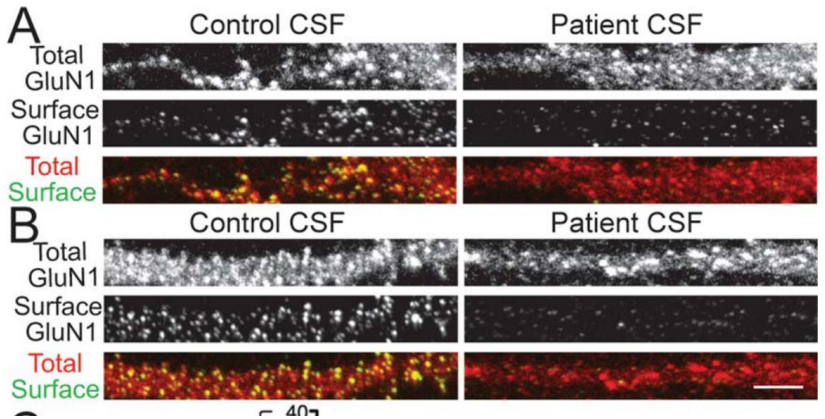
	ADENOVIRUS	AAV	γ-RETROVIRUS	LENTIVIRUS
SIZE	~90-100 nm	~25 nm	~80-100 nm	~80-100 nm
GENOME	dsDNA	ssDNA	ssRNA	ssRNA
PACKAGING CAPACITY	~8 kb – 36 kb	~4.7 kb	10 kb	8 kb
TRANSDUCTION	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High	Moderate	Moderate	Moderate
INTEGRATION	Non-integrating	Non-integrating	Integrating	Integrating
EXPRESSION	Transient	Transient or stable	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-1	BSL-2	BSL-2
IMMUNOGENICITY	High	Low	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*In vivo*	*In vivo*	*Ex vivo*	*Ex vivo*

Discussion

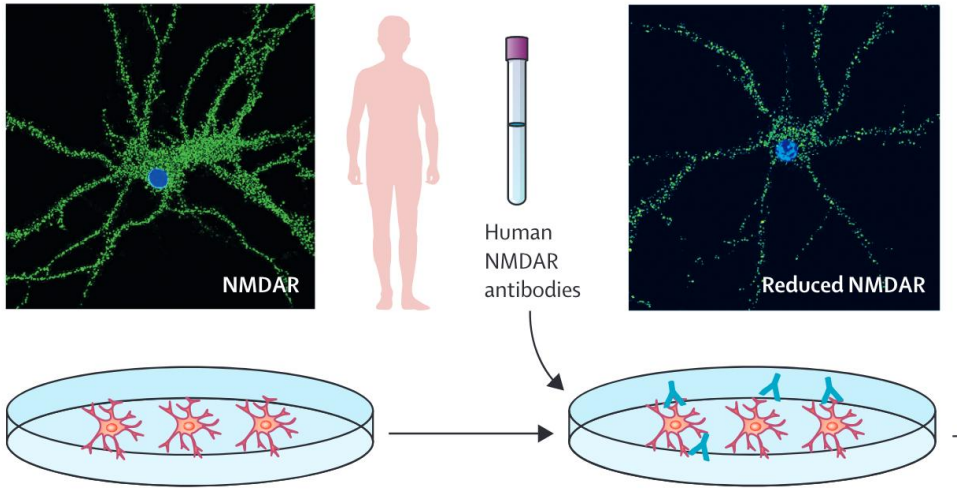
- **New methods of drug delivery**

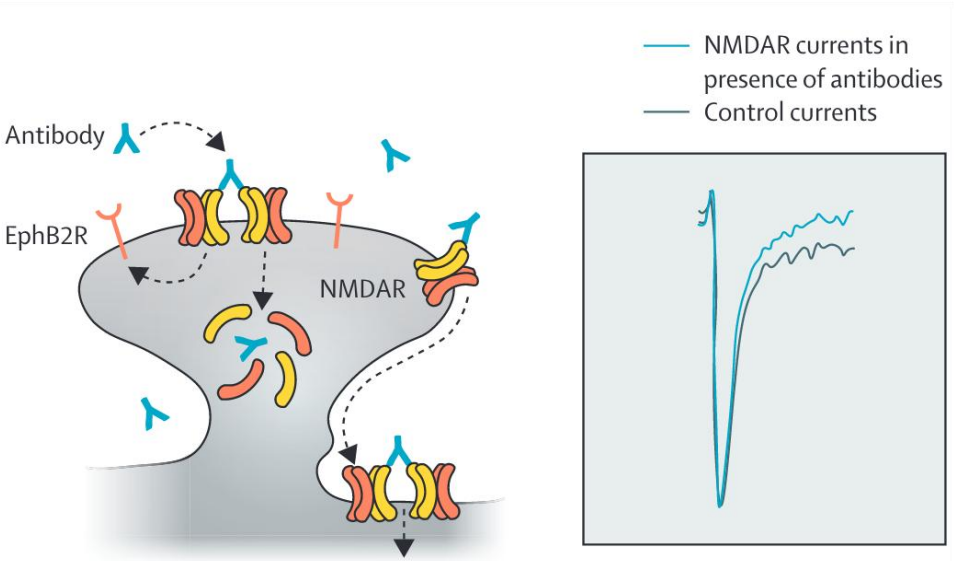
Anti-NMDA receptor encephalitis

Phage coat proteins mimic scFv and achieve drug delivery by inducing receptor internalization



Moscato et.al, 2014





Dalmau et.al, 2019


Q&A

谢谢大家

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