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Screening of CDK inhibitors in breast cancer

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PURPOSE

A new collaborative report from IARC. There were almost 20 million new cases of cancer and close to 10 million deaths from cancer in 2022. Despite an estimated increase to more than 2.31 million new cases, breast cancer became the second most common cancer type, after lung cancer. The most common subtype, hormone-receptor-positive/HER2-negative (HR+ or ER+/HER2-), accounts for 69% of all cases [1]. Cyclin-dependent kinase (CDK) 4/6 inhibition in combination with endocrine therapy is the standard-of-care treatment for patients with advanced-stage HR+HER2- breast cancer [2]. Cell cycle regulators with promising clinical potential include CDK2, CDK4, CDK7, PLK4, WEE1, PKMYT1, AURKA and TTK. Novel inhibitors of these targets, alone or in combination, may overcome CDK4/6 inhibitor resistance [3]. Based on this, we have established a series of in vivo and in vitro assays on CDK family targets. On this basis, we have established a series of in vitro and in vivo experiments on CDK family targets, which can achieve high-throughput screening in vitro experiments, providing a faster screening strategy for CDKs inhibitors.

METHOD(S)

CDK family targets kinase activity assay: Expressing and purifying CDK protein complexes using insect expression systems. The targets are optimized according to biochemical assay optimization procedure, including protein titration and time course, substrate Km testing, protein re-titration used substrate concentration at Km, DMSO tolerance & Z factor test and IC50 testing of commercial inhibitors.

Cell Proliferation Assay: After treating cell lines with inhibitors, medium and CellTiter-Glo reagent mixed 1:1. Incubated 30 mins at RT, fluorescence measured with BMG Labtech.

Construction of T-47D resistant cell line: IC50 concentration of drug is added to cell culture, and the drug concentration is continuously increased when the cells grow to 90% fusion. The cells are still in a stable growth state after 4 weeks of without treatment. Finally, cell proliferation was tested in both resistant and parental cell lines after drug treatment.

BaF3-drived cell line construction by Lentivirus infection: CDK6 plasmids labeled with puromycin were transfected into BaF3 cell lines through lentiviral infection and stable cell line was obtained through resistance screening and removal of IL-3.

CDK NonoBRET Target Engagement assay: establish stable and reproducible assay by optimizing cell density and the ratio of transfection reagents and plasmid. Finally, test the IC50 of the tool inhibitor.

Phosphorylation detection : OVCAR-3 cells were stimulated overnight with 1mM Hdroxyurea and then treated with inhibitors. After cell lysis, pRb was detected using AlphaLISA Surefire Ultra Human Phospho-Rb kit.

FACs assay: OVCAR-3 cells were collected after being treated with inhibitors. Fixed with 75% alcohol and stained with Propidium Iodide.

Western Blot detect CDK protein: optimizing assays using high-throughput JESS instrument.

Construction of CDX model: Breast cancer cells with different cell densities are injected subcutaneously to monitor the tumor volume regularly. If the tumor volume shows a linear increase and the weight of mouse does not show significant reduction, the modeling is successful.

RESULT(S)

In Vitro: We obtained high purity CDK/Cyclin protein and established biochemical assays. The IC50 values of inhibitors on corresponding targets are all at the nanomolar range, which is close to the literature reports (Fig1).

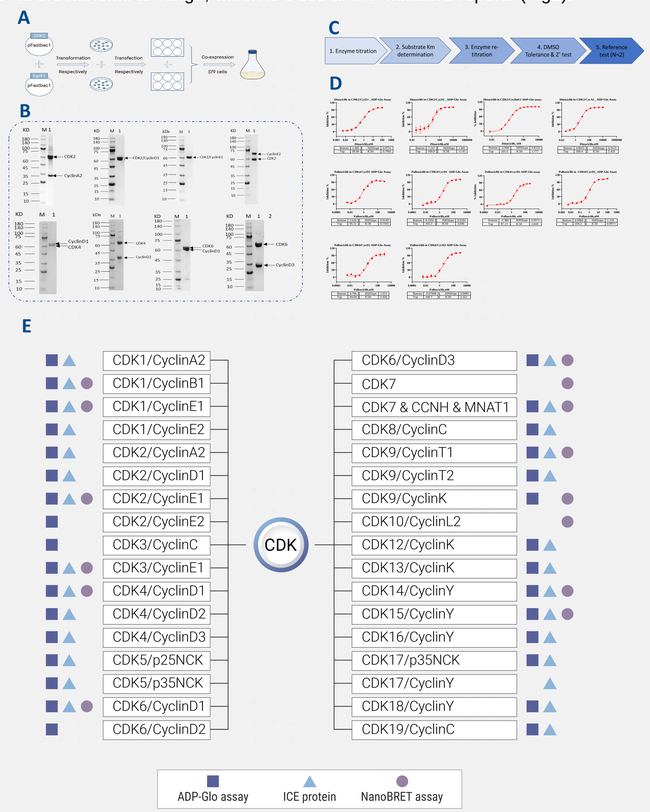

Figure1. A. Procedure for CDK2,4,6 protein complex expression. B. SDS-PAGE for CDK2,4,6 protein complex purification. C. Procedure for biochemical assay set up. D. The results of commercialized inhibitors in CDK2,4,6 biochemical assays. E. In vitro enzymatic and cellular assays of the CDK family that has been constructed.

We have constructed cellular assays for stable drug screening. Stable BaF3-CDK6 and T-47D/ Palbociclib R cell lines have obtained (Fig2).

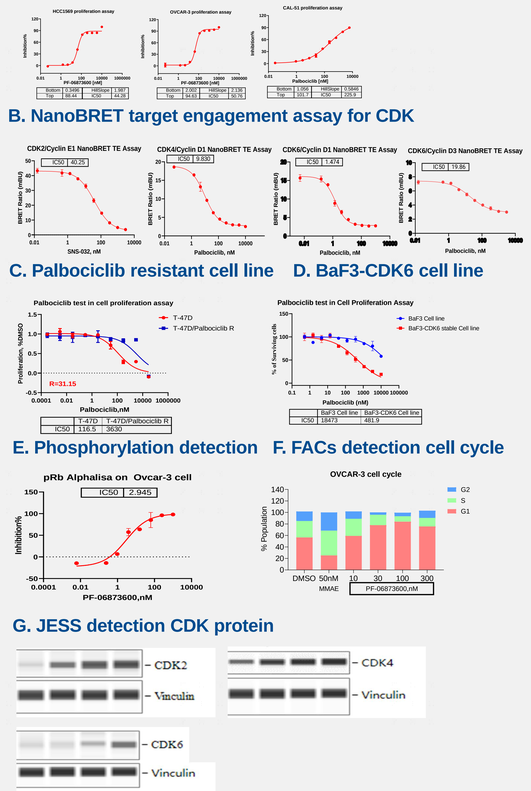

Figure2. A. Result of commercial inhibitor in Breast cancer cell proliferation assays; B. Inhibitor IC50 test of inhibitor in NanoBRET assay; C. Palbociclib IC50 testing in stable palbociclib resistant T-47D and T-47D breast cancer cell lines; D. Palbociclib IC50 testing in BaF3-CDK6 derived cell line E. Phosphorylation Rb detection in Alpha Lisa assay after PF-06873600 treatment; F. FACs detect cell cycle after Ovcar3 cell by PF-06873600 treatment. G. Western blot detect CDK protein expression in MDA-MB-231 cell by JESS;

RESULT(S)

In Vivo: Through optimization, we obtained a linear growth curve of tumor volume and no significant reduction in mouse weight. CDX model of breast cancer with four cell lines successfully constructed (Fig3).

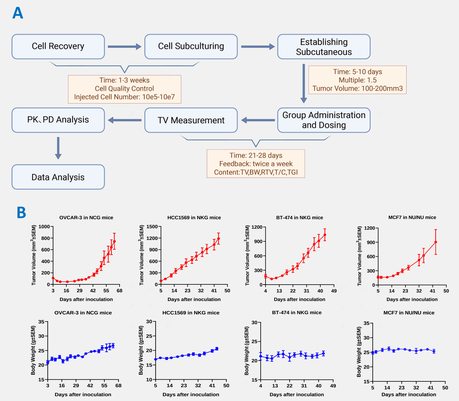

Figure3. A. Procedure for animal tumor model set up and PK/PD analysis. B. The results of Breast cancer cell line CDX model and animal body weight.

CONCLUSION(S)

In conclusion, inhibition of CDK targets could be regarded as a rational approach in Breast cancer. we constructed an experimental cascade from in vitro to in vivo, which is composed of protein production, biochemical assays, cell line construction, cellular assays, and animal modeling. Our CDK screening cascade can satisfy the mechanism study of CDK as well as efficient and comprehensive screen of CDK inhibitor, thus accelerate the novel drug discovery.

REFERENCE

[1] U.S. Cancer Statistics Data Visualizations Tool CDC.2023 June 9; https://www.cdc.gov/cancer/uscs/dataviz/index.htm

[2] Laura Morrison, Sibylle Loibl & Nicholas C. Turner .The CDK4/6 inhibitor revolution a game-changing era for breast cancer treatment. Nature Reviews Clinical Oncology volume 21, pages89–105 (2024).

[3] Jesús Fuentes-Antrás. Seize the engine: Emerging cell cycle targets in breast cancer. Published online 2024 Jan 24. doi: 10.1002/ctm2.1544

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