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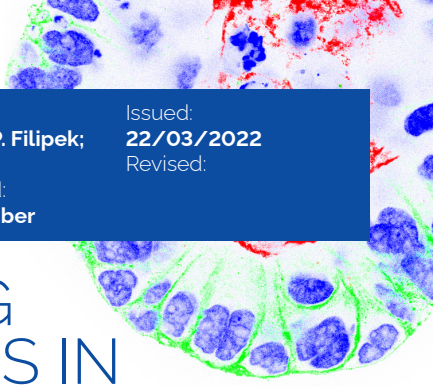

SOP –ADSI-4.0

Author:
R. Gstir; P. Filipek; D. Lesiak
Approved:
Lukas Huber

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DRUG-SCREENING OF CDK INHIBITORS IN BREAST CARCINOMA CELLS

Purpose

The SOP-ADSI-4.0 was issued to describe how to perform drug screenings of cyclin-dependent kinase inhibitors (CDK inhibitors) in breast carcinoma cells with different invasive capacities and radiation sensitivities.

Scope

The SOP-ADSI-4.0 includes the generation of radioresistant and invasive clones from AU565 and MDA-MB-231 breast carcinoma cells. This SOP also includes handling and general cell culture techniques in advance to the drug screening experiment, as well as the drug screening experiment itself.

Introduction

The world Health Organization (WHO) reports worldwide 2.1 million women with breast cancer. Especially metastatic progression and radioresistance of breast carcinoma constitute a major problem in clinical oncology. It is assumed that metastasis is responsible for most breast-cancer related deaths, which are 15 % of all cancer-related mortalities. Metastasis cascade contains a number of steps such as cancer cell invasion in surrounding tissues followed by cell intravasation into the blood and/ or lymphatic vessels, extravasation into the tissue of distant organs, and, finally, colonization and growth in the targeted organs. PCare serves as a technological platform to overcome tumor resistance to therapies against cancer. The platform addresses beside lung and colon carcinoma, also breast cancer with a high incidence in the program region (Report AIOM AIRTUM 2018 und Statistik Austria). In this study the P-Care project partners aim to develop methods to find active drug substances to break through resistance of cancer cells against up-to-date therapies. Cultivation of cancer cells in presence of chemotherapeutic substances serves as a future strategy to generate chemoresistant cancer cell lines.

Weekday	Day	Media	Timepoint
Monday	1	Cell seeding	-
Tuesday	2	Cell treatment with CDK inhibitors at indicated doses	0 h
Wednesday	3	Sample collection for cell viability and cell proliferation assay	24 h
Thursday	4	Sample collection for cell viability and cell proliferation assay	48 h
Friday	5	Sample collection for cell viability and cell proliferation assay	72 h

Table 1. Time schedule for drug testing

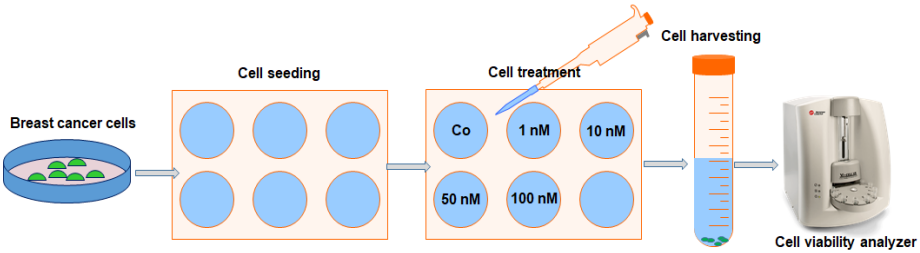

Figure 1. Schematic procedure – Drug-screening of CDK inhibitors in breast cancer cells

1. Reagents, solutions, and cell culture media

1.1 Reagents

- RPMI 1640 (#31870-074, Gibco, Thermo Fisher Scientific)
- DPBS, 1 x no calcium, no magnesium (#P04-36500, PAN-Biotech)
- Penicillin-Sterptomycin-Glutamine (100x) (#103870-074 Gibco, Thermo Fisher Scientific)
- Trypsin-EDTA Solution (x10) (#T4174, Sigma-Aldrich, Millipore)
- Research Grade Fetal Bovine Serum heat-inactivated (#12350273, Fisher Scientific)
- Insulin Solution from bovine pancreas (#I0516-5ML, Sigma-Aldrich, Millipore)
- Trypan Blue Solution (#T8154, Sigma-Aldrich, Millipore)

- Cell WASH (#349524, BD Biosciences)
- Coulter CLENZ® Cleaning Agent (#8448222 Beckham Coulter)
- Isopropanol (C3H8O) 70% (#CN09.3, Carl Roth)
- Palbociclib
- Abemaciclib
- Otviciclib
- Dinaciclib

1.2 Compositions

- **Cell Culture Medium for MDA-MB-231 and Au565 breast carcinoma cells**

500 mL RPMI 1640

5 mL Penicillin-Sterptomycin-Glutamine (100x)

50 mL of FBS

Store at 4 °C

- **Cell Culture Medium for T47D breast carcinoma cells**

500 mL RPMI 1640

5 mL Penicillin-Sterptomycin-Glutamine (100x)

50 mL of FBS (10% final concentration)

0,2 Units/mL bovine insulin

Store at 4 °C

2. Equipment

- ViCell Xr cell viability analyzer (Beckman Coulter)
- Elekta Precise Linear Accelerator (Elekta Oncology Systems, UK)
- Incubator Hera-Cell 150 (Thermo Scientific)
- Laminar Air-Flow Steril-Antares
- Centrifuge (4°C) Eppendorf 5810R
- Microscope Leica DMIL-LED
- Vortex Mixer (VWR)
- Waterbath Biosan-PeqLab
- Neubauer Cell Counting Chamber
- Uncoated 8 µm-pore membrane in Boyden chamber (Corning Life Sciences)
- Polystyrene box
- Box with crashed ice
- Pipette Aid
- Set of pipettes 20 µL, 200 µL, 1000 µL and pipette tips

3. Plastics

- Gewebekulturflaschen, 250 mL Greiner 658175
- 6-well-plates (Greiner bio-one # 657160)
- 50 mL Polypropylene Conical Tube (#Falcon 352070)
- 15 mL Polypropylene Conical Tube (#Falcon 352096)
- Sterile 0,5 mL, 1,5 mL, 2mL and 5 mL tubes
- Pipettes 1 mL, 5 mL, 10 mL, 25 mL, 50 mL

4. Generation of radioresistant breast carcinoma cells

4.1 Preparation of material and reagents

- Prepare and pre-warm cell culture medium with and without 10 % FBS for MDA-MB-231, AU565 or T47D respectively
- Cultivate cells to obtain 5x105 per 6-well

4.2 Procedure

- Add 2.5 mL of cell culture medium with 10 % FBS (chemoattractant) into wells of a 6-well-plate.
- Place Boyden Chamber into wells and add 2.5 mL of serum-free cell culture medium on 8 µm pore membrane of Boyden Chamber.
- Collect migrated cells on surface of 6-well-plate every second week.
- Seed the collected cells again into Boyden Chamber.
- Repeat this procedure ten times to generate invasive cell lines.

5. Generation of invasive breast carcinoma cells

5.1 Preparation of material and reagents

- Prepare and pre-warm cell culture medium with 10 % FBS for MDA-MB-231, AU565 or T47D respectively

5.2 Procedure of cell seeding and drug treatment

- Seed 10^5 cells in 3 ml medium per well in 6-well plates.
- Irradiate cells every 2nd week with 10 Gy 16 MV x-rays using an Elekt Precise Linear Accelerator at a dose rate of approx. 1.8 Gy/min.
- Repeat until total dose of 100 Gy. Collect survived cells for later experiments as radioresistant. The newly received cell lines maintain resistance to ionizing radiation independently from passage number

6. Seeding of breast carcinoma cells into 6-well-plates

6.1 Preparation of material and reagents

Harvest breast carcinoma cells after expansion from 250 ml cell culture flasks using trypsin solution.

- Prepare and pre-warm DPBS and required supplemented culture media
- 50 mL conical tubes
- Pipette tips and pipettes (10µl, 200µl, 1000µl)

6.2 Procedure of cell seeding and drug treatment

- After cell washing in DPBS, resuspend the cells in 5 mL medium, count the cells and seed 105 cells in 3 ml medium per well in 6-well plates.
- Incubate the cells at 37 °C, 5 % CO2 and 95 % r.H. for 24 hours
- After 24 h of incubation, carefully remove medium and wash the cells with DPBS in each well, add appropriate concentrations of CDK inhibitors diluted in 4 mL medium
- Incubate at 37 °C, 5 % CO2 and 95 % r.H. for 72 hours

6.3 Measurement

- Untreated and treated cells are detached from the bottom of wells in 6-well plates.
- Harvested cells are washed with DPBS, and cell pellet is resuspended in RPMI 1640 medium without supplements.
- Resuspended cells are placed into the ViCell inserts
- Open ViCell program, give the name of each measurement and aquire the cell count in each sample (total cell number, viable cell number and cell viability)
- Export the data as EXCEL file for further calculations and evaluation of the received results.

7. References

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NOTES