Characterization of Resistance to CDK4/6 Inhibitors and Endocrine Therapy in Invasive Lobular Breast Cancer Cell Lines

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Background: ~10-15% of breast cancers are invasive lobular carcinoma (ILC) and are estrogen receptor (ER) – positive (+) with a loss of E-cadherin and are harder to detect than invasive ductal carcinoma (IDC). Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors have become a new standard of care for patients with ER+ breast cancer. Herein, we discover mechanisms of resistance and identify strategies to overcome them.

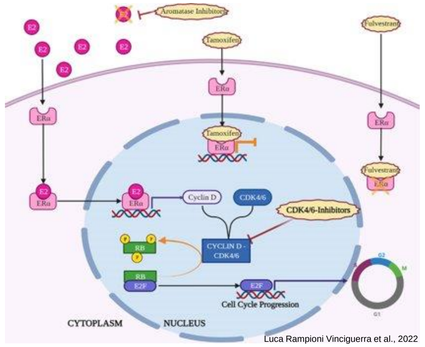
Methods: To compare ILC to IDC, we generated cell lines resistant to CDK4/6 inhibitors and endocrine therapies and performed RNA-Seq with functional analysis.

Results: The ILC and IDC models of CDK4/6 inhibitor resistance exhibited hallmark signature pathways involved in cell signaling. The ILC cell lines exhibited differences in epithelial mesenchymal transition (EMT) markers, androgen receptor (AR), ERBB2/HER2 pathway, and multidrug resistance (MDR) proteins.

Conclusions: We confirmed previously reported hallmark pathways as well as discovered novel pathways of resistance in ILC cells.

INTRODUCTION

- ~10-15% of breast cancers are invasive lobular carcinoma (ILC).
- Limited ILC Cell Line Models are available for research.
- Endocrine treatment options include: Aromatase Inhibitors, Tamoxifen, Fulvestrant.
- Treatment with cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) include: Palbociclib, Abemaciclib, Ribociclib.



- Mechanisms of resistance to CDK4/6i: RB1 loss, CDK amplification, Estrogen receptor /Progesterone Receptor loss, Androgen Receptor overexpression, and JAK/STAT signaling dysregulation.
- Cell Lines: MDA-MB-134, SUM44PE, MCF-7, T47-D, MDA-MB-361, BT474, ZR-75-1

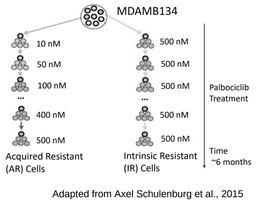
OBJECTIVE

Generate cell line models in order to uncover mechanisms of resistance to CDK4/6 inhibitors and endocrine therapy and identify strategies to overcome resistance.

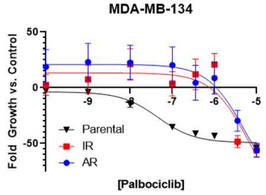
ESTABLISHING RESISTANCE CELL LINES

PALBOCICLIB RESISTANCE MODELS

Cells were treated for >6 months with palbociclib to select for resistant subclones.

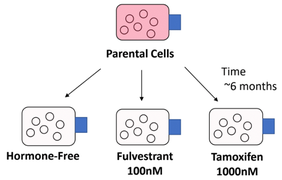


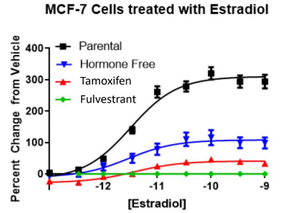
Adapted from Axel Schulenburg et al., 2015



ESTROGEN RESISTANCE MODELS

Cells were deprived of estrogen and treated with fulvestrant or tamoxifen for >6 months to select for resistant subclones.





HALLMARK PATHWAYS

The ILC and IDC models of CDK4/6 inhibitor resistance exhibited significant hallmark signature pathways involved in proliferation.

RNA-Seq identified significant proliferative pathways in the short term treatments. The longer term treatments identified Epithelial Mesenchymal Transition (EMT), and estrogen response gene sets.

3-Day Treatment
E2F Targets
G2M Checkpoint
Mitotic Spindle

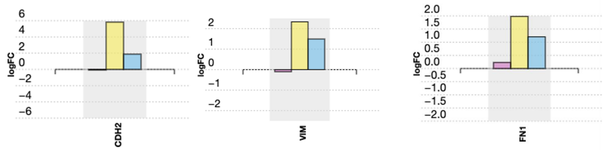
IR 500
Estrogen Response Late
Epithelial Mesenchymal Transition
G2M Checkpoint
Estrogen Response Early
Hedgehog Signaling
KRAS Signaling

AR 100
E2F Targets
G2M Targets
Mitotic Spindle
Epithelial Mesenchymal Transition
Estrogen Response Late

EMT MARKERS

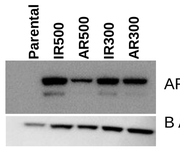
SUM44PE, treated with Palbociclib upregulated hallmarks of epithelial mesenchymal transition (EMT) markers: CDH2 (N-Cadherin), VIM (vimentin), FN1 (fibronectin).

Examining IR (yellow bars), and the AR cells (blue bars) we observed a significant upregulation of these EMT markers compared to the parental cells (pink bars). These data suggest an EMT mechanism of resistance to Palbociclib in ILC.

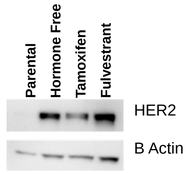


AR & HER2 UPREGULATION

MDA-MB-134 cells treated with Palbociclib upregulated androgen receptor (AR) while cells maintained in endocrine therapy conditions upregulated HER2.



Palbociclib Conditions



Endocrine Conditions

CONCLUSIONS AND FURTHER DIRECTIONS

- We generated breast cancer cell line models of ILC and IDC resistant to CDK4/6 inhibitors or endocrine therapies.
- RNA-Seq identified previously reported hallmark pathways as well as discovered novel pathways of resistance in ILC cells including: EMT pathways, AR, and HER2.
- Ongoing studies include examining significant genes at the protein level as well as targeting these genes with drugs in order to reverse resistance.

REFERENCES AND FUNDING

- Axel Schulenburg et al., 2015
- Huang et al., 2022
- Luca Rampioni Vinciguerra et al., 2022
- Rasha et al., 2021

Dynami Foundation

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