Targeting Breast Cancer Resistance to Palbociclib via Oncolytic Virotherapy

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Introduction

Palbociclib is a first-in-class inhibitor of cyclin dependent kinases 4 and 6 (CDK4/6) and is the new standard of care for metastatic estrogen receptor-positive (ER+) breast cancer (BC). Although palbociclib markedly improves progression-free survival in this patient subset, metastatic BC remains incurable.

Unfortunately, 45% of treated patients fail to respond to palbociclib (intrinsic resistance) and 50% of those who initially respond develop resistance and relapse after two years of therapy (acquired resistance). Accordingly, there remains an urgent unmet need to develop effective therapies for the treatment of ER+ stage IV breast cancer.

We and others found that autophagy and cyclin E overexpression play a key for in the BC resistance to palbociclib. Interestingly, autophagy and cyclin E expression are important factors for an efficient oncolytic adenovirus replication and oncolysis. Therefore, we hypothesize that palbociclib-induced autophagy and cyclin E expression could enhance oncolytic virotherapy efficacy.

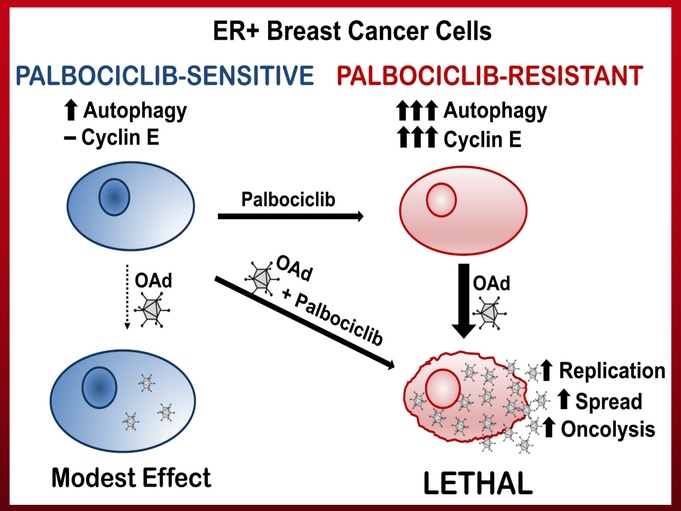

Figure 1. Rationale for the Proposed Therapeutic Approach. Palbociclib resistant breast cancer cells display increased levels of autophagy and cyclin E, rendering them selectively susceptible to OAd-mediated oncolysis. Palbociclib increases replication and anti-tumor activity of the OAd in sensitive and resistant cells.

Results

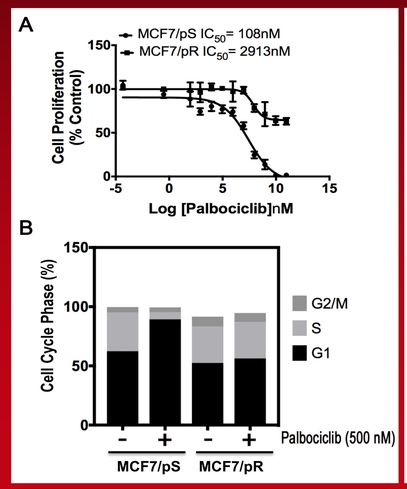

Figure 2. Characterization of MCF7pR cells. (A) Cell viability was measured using a FluoReporter assay after 72 hours of palbociclib exposure. Inhibition of proliferation % was calculated as a function of the number of cells compared to control. Each data point represents the average of three independent experiments; **(B)** Cells were treated with 500nM palbociclib for 24 hours followed by Flow cytometry cell cycle analysis.

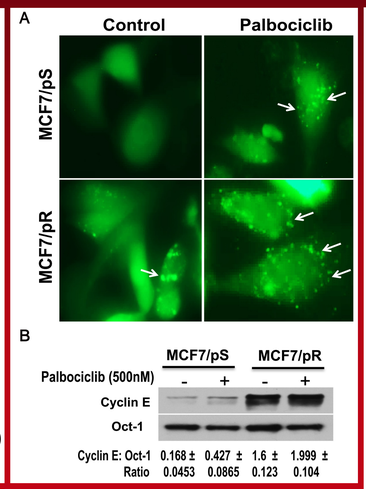

Figure 3. MCF7 cells resistant to palbociclib display increased autophagy and cyclin E levels. (A) Cells were transfected with a pEGFP-LC3 plasmid and treated with either vehicle control (0.5% water) or 500nM palbociclib for 24 hours. Formation of autophagosomes is depicted by punctate structures (arrows); (B) Nuclear extracts were analyzed for Cyclin E and Oct-1 expression by western blot (top) and densitometry was measured using the UN-SCAN-IT gel 5.3 program (bottom).

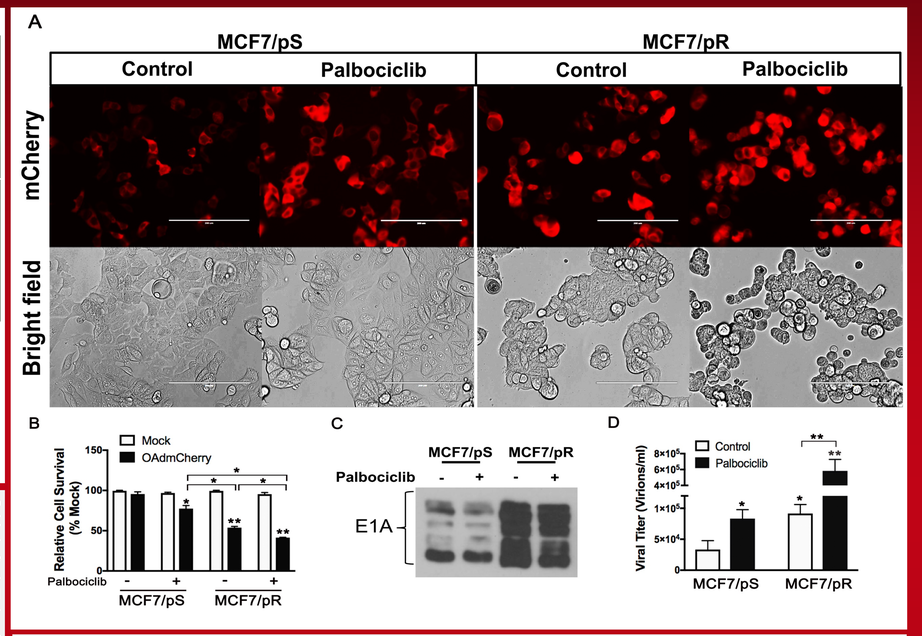

Figure 4. MCF7 cells resistant to palbociclib display increased OAdmCherry-mediated CPE. (A) Cells were infected with an OAd at a multiplicity of infection (MOI) concentration of 5 +/- palbociclib (500 nM). Expression of mCherry as a surrogate for viral infection was evaluated by fluorescence microscopy 48 hours post-infection; (B) Crystal violet staining was used to determine cell viability and is expressed as percent (%) of mock transfected vehicle treated cells.; (C) Expression of adenovirus E1A protein was determined by western blot analysis; (D) Release of infectious viral particles to the media was measured using the 50% end-point dilution method, also known as the 50 % tissue culture infectious dose ($TCID_{50}$) assay. * p value < 0.05, ** p value < 0.005. When not indicated, significance was determined against OAdmCherry control treated cells.

Conclusions

- We stablished a novel ER + breast cancer cell line resistant to palbociclib facilitating thus our understanding of the mechanism by which BC acquire resistance to palbociclib.
- Our studies demonstrate *for the first time* that an OAd, used as monotherapy or in combination with palbociclib, causes significant cytoxicities in both palbociclib-sensitive and palbociclib-resistant ER+ breast cancer cells.

Further Direction

We will further evaluate this new therapeutic strategy in both palbociclib-sensitive and palbociclib-resistant triple-negative breast cancer cells to demonstrate the its reproducibility in other BC cell type and in relevant tumor-bearing models. For our *in vivo* studies, we will use the mouse intraductal (MIND) model.

Acknowledgements

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