Evaluation of oridonin derivatives with Protacs against triple-negative breast cancer

Gabrielle Vontz, Doerte Raphi Fricke, Ruixia Ma, Junhai Xu, Qiang Shen

Department of Interdisciplinary Oncology
Stanley S. Scott Cancer Center
School of Medicine
Louisiana State University Health Sciences Center - New Orleans

Introduction

Natural products have served as molecular starting points to develop therapeutic agents. Oridonin, a natural kaurene-type diterpenoid enriched in the traditional Chinese medicinal herb *Rabdosia rubescens*, has been used as a backbone molecule to derive potential anticancer drugs. Oridonin derivative compound CYD0618 was identified as a potent anticancer agent inhibiting cancer growth both *in vitro* and *in vivo*[1]. CYD0618 was shown to directly bind and inhibit STAT3, a transcription factor responsible for a signaling cascade that is constitutively active in ~70% of human cancers, including breast cancer. Recently, PROteolysis TArgeting Chimera (PROTAC) technology has been developed to induce proteasomal degradation of drug targets[2]. PROTAC modifications of experimental STAT3 inhibitors have been explored to improve potency through STAT3-specific degradation; however, none has reached clinical phase applications. Thus, in an attempt to improve anticancer activity of CYD0618, our group has designed several novel CYD0618-based derivatives containing PROTAC modifications. In this project, we characterize the anticancer properties of these CYD0618-PROTAC derivatives by testing their effects on proliferation, colony formation, and apoptosis in a triple-negative breast cancer cell line, MB-MDA-231.

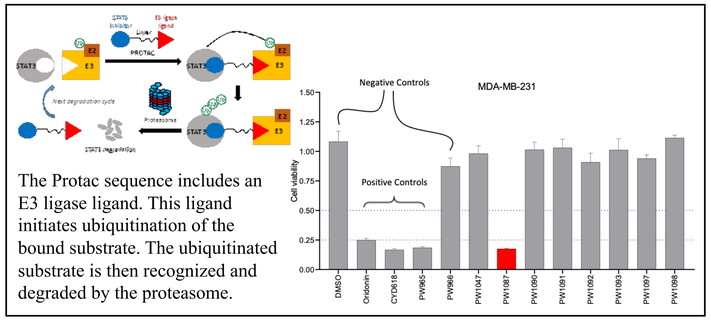
Methods

Proliferation: Proliferation was assessed using an MTT assay. In brief, viable cells reduce a substrate to a purple product which can be measured by colorimetry. Thus, MTT assays can be used to measure viable cells after a treatment. An initial MTT screen was performed in which MB-MDA-231 cells were seeded, treated with 10 μM of compound for 72 hr., and viable cells were measured and compared to a DMSO control. From this screen, PW1087 was chosen for further characterization. Subsequent MTT assays were performed in which cells were seeded, treated with a DMSO control, 0.1μM, 0.33 μM, 3.33 μM, and 10 μM of each compound. From this data, the IC_{50}, the concentration at which a compound reduces viability by 50%, was calculated for each compound.

Colony formation: Cells were seeded, treated with a single dose of the labeled concentration of compound, and incubated. After 7 days, colonies were visualized and quantified by fixing with paraformaldehyde and staining with crystal violet.

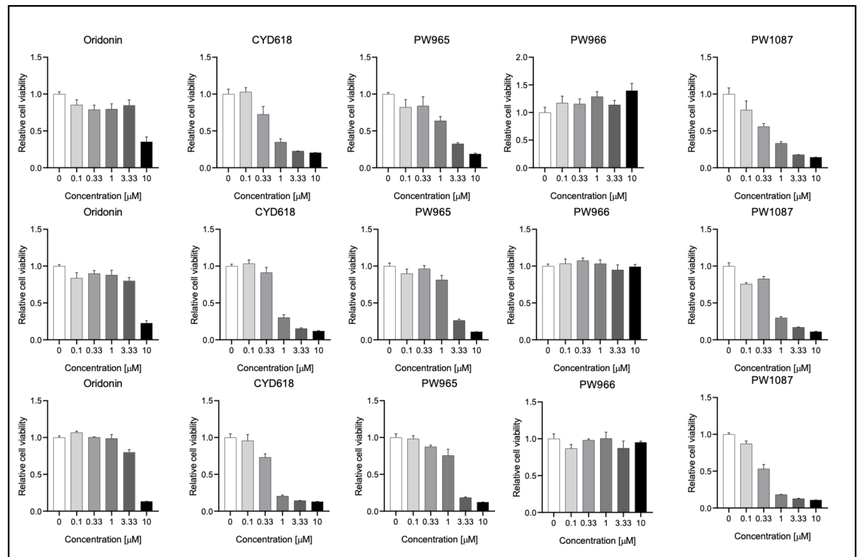
Apoptosis: Apoptotic cell populations were analyzed using the Muse Annexin V & Dead Cell Kit (Luminex). In brief, cells were seeded, treated with 5 μM of the indicated drug, and collected after 24 hr. or 48 hr. of treatment. Both floating (dead) as well as adhered cells were included in the analysis. The Muse Annexin V protocol was followed to measure live, early apoptotic, late apoptotic, and dead cells. In principle, the kit uses fluorescence to measure 1) annexin V binding to phosphatidylserine, a phospholipid that is externalized during apoptosis and, 2) a dead cell marker.

Initial Screen



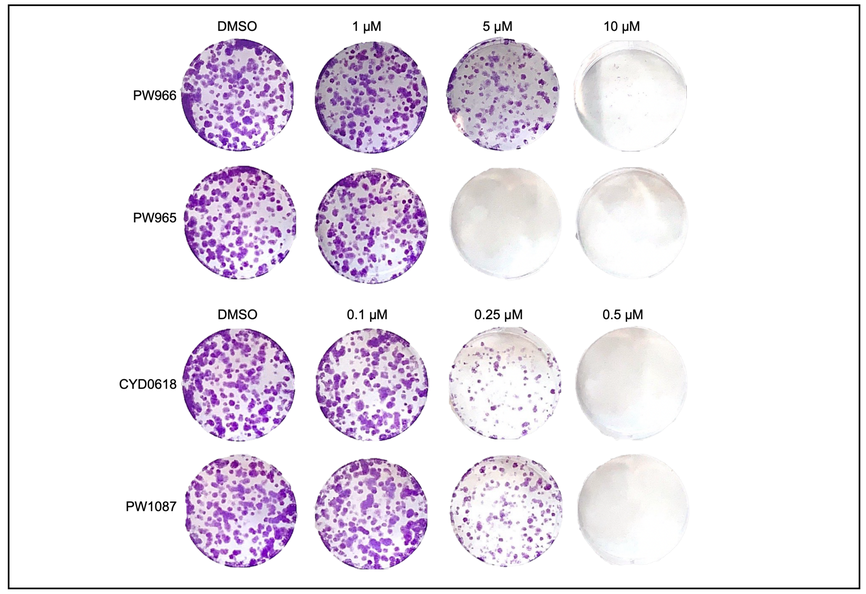
The Protac sequence includes an E3 ligase ligand. This ligand initiates ubiquitination of the bound substrate. The ubiquitinated substrate is then recognized and degraded by the proteasome.

Proliferation

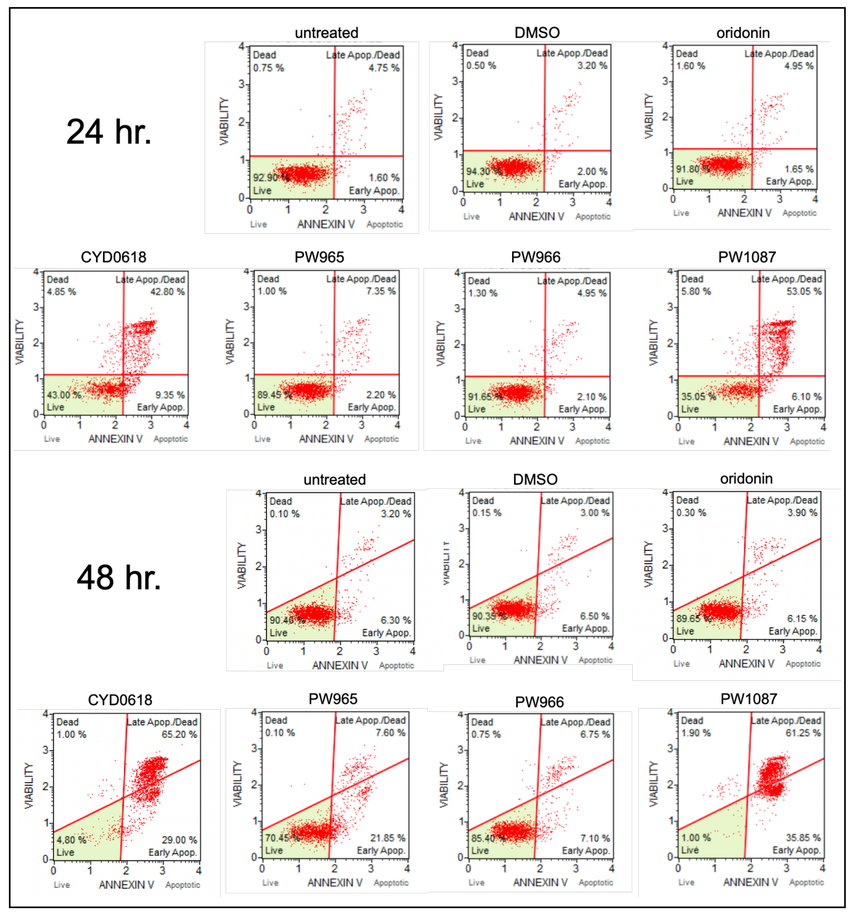


MDA-MB-231	Oridoin	CYD618	PW965	PW966	PW1087
1st	9.269	0.8311	1.672	~ 0.000	0.4623
2nd	5.865	0.7512	2.056	532.1	0.6723
3rd	5.307	0.5467	1.676	2.2E-09	0.3738
IC50	6.81 ± 1.24	0.71 ± 0.08	1.80 ± 0.13	177.37 ± 177.37	0.50 ± 0.09

Colony formation



Apoptosis



Conclusions & Future Directions

From our initial MTT screen, PW1087 was identified as a potential anticancer agent. Proliferation, colony formation, and apoptosis experiments show PW1087 has comparable if not greater potency than parent compound CYD0618 against MB-MDA-231 triple-negative breast cancer cells. Thus, PW1087 shows potential as a potent anticancer agent that utilizes Protac technology to target STAT3.

Future experiments intend to further characterize CYD0618-Protac derivatives by assessing effects on cellular motility, determining molecular interactions with STAT3, and testing antigrowth activity on tumor xenografts.

References

1. Shen, X. *et al.* A thiazole-derived oridonin analogue exhibits antitumor activity by directly and allosterically inhibiting STAT3. *J. Biol. Chem.* **294**, 17471–17486 (2019).
2. Zou, Y., Ma, D. & Wang, Y. The PROTAC technology in drug development. *Cell Biochem. Funct.* **37**, 21–30 (2019).

This research project was supported through the Stanley S. Scott Cancer Center, Dept. of Interdisciplinary Oncology, School of Medicine, LSU Health Sciences Center