Screening and validation of FDA approved oncology drugs for induction of progesterone receptor expression in endometrial cancer cells

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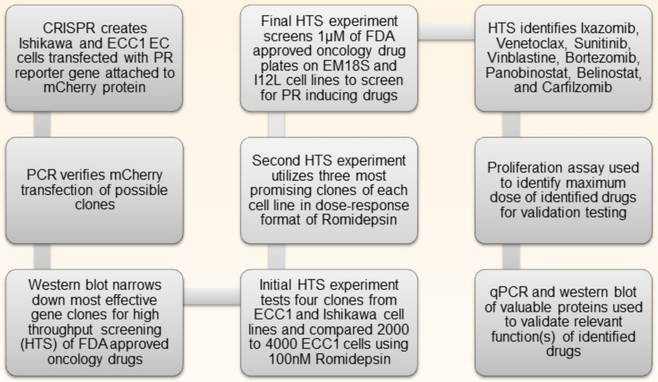
Introduction

Endometrial cancer (EC) is the most predominant uterine cancer and continues to grow in incidence and deaths annually. A characteristic of malignant endometrial tumors is sensitivity to the growth **promoting** effects of estrogen and the growth **limiting** effects of progesterone. As such, hormonal therapy with progesterone, or its analog progestin, is typically used in EC therapy; however, it is not lasting in efficacy due to the observed downregulation of progesterone receptor (PR) expression in cancer cells. While this observation presents multiple areas for study, this investigation pursues the screening and validation of existing FDA-approved anticancer drugs which induce PR and mCherry, an endogenous PR reporter gene which faithfully reflects PR expression in endometrial cancer cells in real time. Initial analyses utilize Romidepsin, an FDA approved histone deacetylase (HDAC) inhibitor, which is proven to induce PR expression in EC gene clones.

Objectives

- Identify ideal ECC1 and Ishikawa gene clones to visualize PR induction in high throughput screening, western blot, and qPCR analyses
- Screen for FDA-approved oncology drugs and ideal doses for induction of PR expression
- Validate identified drugs for PR induction

Materials and Methods



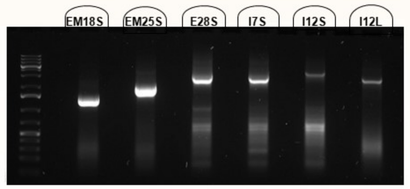


Figure 1. Junction PCR of genomic DNA for verification of gene insertion. Bands identify location of PR reporter gene in the clone, integrated gene clones are selected. **EM18S:** homozygous; EM25S: homozygous; E28S: heterozygous; I7S: heterozygous; I12S: heterozygous; **I12L**: homozygous. These genes are used in the first round of HTS.

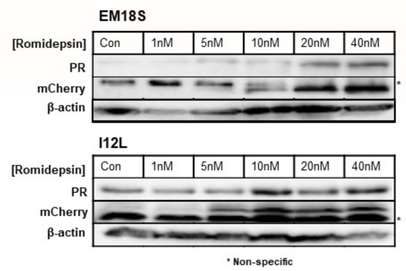


Figure 2. Western blots showing the dose response of gene clones EM18S (of the ECC1 cell line) and I12L (of the Ishikawa cell line) to Romidepsin at concentrations 0-40nM. 12% gel used for mCherry and β-actin and 7.5% gel used for PR; both utilized a wet transfer. The steady increase in band size according to dose indicates that Romidepsin is successfully inducing PR and mCherry expression in EC cells. β-actin, the loading control, shows similarity in loading volume throughout.

Figure 3. High throughput screening reading taken at 72 hours following treatment of PR reporter gene clones with 100nM of Romidepsin.

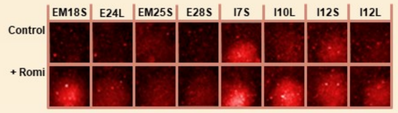


Figure 4. HTS reading taken 72 hours following treatment of PR reporter gene clones with 1:3 dilutions of 0nM-1000nM of Romidepsin.

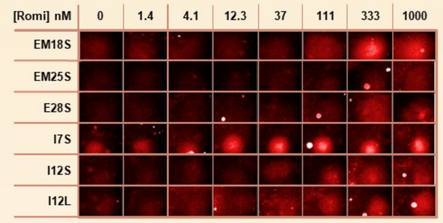
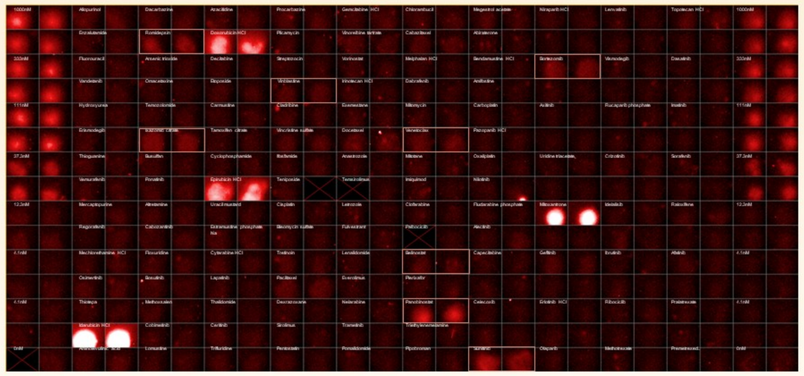


Figure 5. HTS reading taken 72 hours following treatment of EM18S cells with 1μM from FDA-approved oncology drug plates.



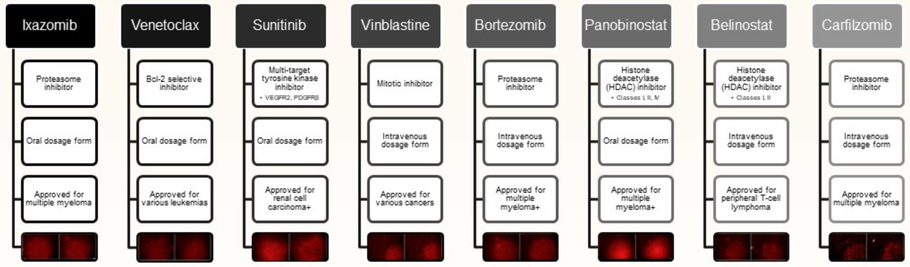


Figure 6. Proliferation assay for dose response of eight identified FDA approved oncology drugs using ECC1 cells. This assay determines maximum doses of drugs before causing cell death to use in initial qPCR and western blots of drugs.

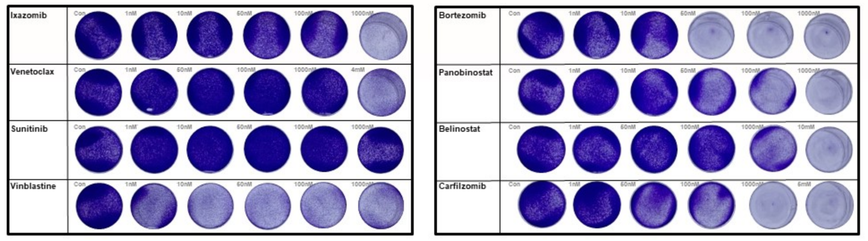
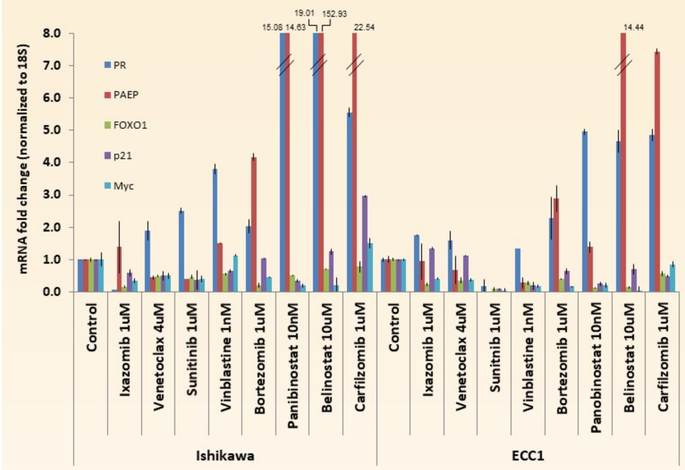
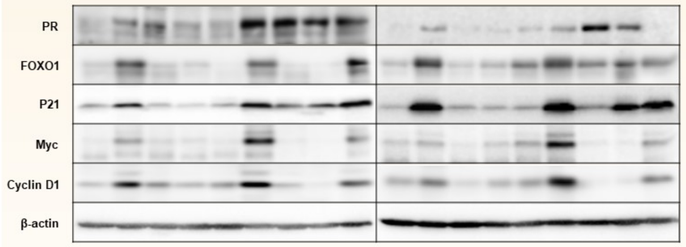


Figure 7. qPCR and western blot data for EC proteins which correlate with upregulation and downregulation of PR expression of 8 identified FDA approved oncology drugs in Ishikawa and ECC1 cells. Ideal responses are upregulated PR, PAEP, FOXO1, p21 and downregulated Myc and Cyclin D1. qPCR fold change identifies drug response compared to control with standard deviation bars.





Conclusions and Future Implications

- Identified valuable gene clones of Ishikawa and ECC1 cell lines for high throughput screening as EM18S and I12L
- Screening identified eight FDA approved oncology drugs as potential small molecule inducers of PR expression in EC cells
- Significant alteration of PR downstream genes seen in Ixazomib, Bortezomib, Panobinostat, and Belinostat
- Initial validation testing did not yield desired results, but further testing may indicate better doses or methodology for ideal induction of PR and other corresponding proteins

Acknowledgments

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