Phytoalexin Glyceollin I Suppresses Viability in HER+ Breast Cancer Cells

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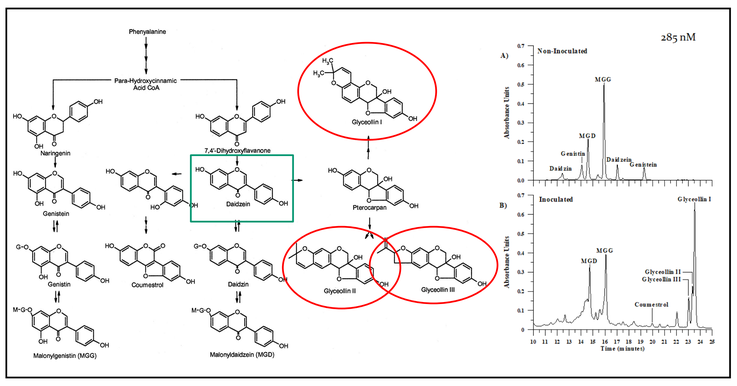
Abstract

For women worldwide, breast cancer (BC) ranks top 2 in both annual quantity of cases and deaths among cancers. Leading these statistics, developing new methods for combating breast cancer remains at the forefront of cancer research. Types of breast cancer are differentiated based on the presence of 3 receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). HER+ BC targeted agents such as trastuzumab, pertuzumab, and lapatinib, alone or in combination with traditional chemotherapeutics, have become the standard of care for this BC subtype, significantly improving patient survival rates. However, the development of resistance to targeted therapy represents a major obstacle in the treatment of HER+ breast cancer, highlighting a critical need to identify novel therapeutic targets to treat resistant HER+ breast cancer.

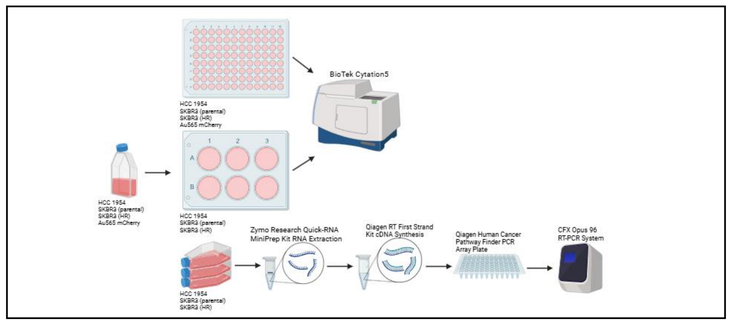
Isoflavonoids, an important class of natural compounds produced from numerous plant sources including legumes, have been identified to be beneficial for human health. A member of the legume family, the soybean, maintains a high isoflavonoid content, specifically daidzein and stress induced derivatives called glyceollins. Recent studies have demonstrated glyceollin activity against ER+ breast cancer due to inhibition of ER and its associated pathways. To date the impact of glyceollins on other breast cancer subtypes has not been fully explored. Here, we utilized a panel of HER+ breast cancer cell lines (HCC 1954, Au565, SKBR3), as well as derived trastuzumab-resistant variants (Herceptin resistant SKBR3), to evaluate the effects of glyceollin treatment, alone or in combination with other targeted agents, on cell viability and clonogenicity. Additionally, we analyzed changes in downstream gene expression using qRT-PCR profiler array for Human Cancer Pathways to define potential glyceollin-targeted pathways involved in the regulation of HER2 breast cancer cell biology.

Results demonstrated that glyceollin was able to decrease cell survival and colony formation across cell lines. Further glyceollin suppressed expression of pro-oncogenic genes in Herceptin-resistant SKBR3 cell lines. Our preliminary findings provide support for a novel approach using isoflavonoids in the development of targeted therapy for HER+ BC.

Glyceollin Biosynthesis



Methods Overview



Reduction of Cell Viability

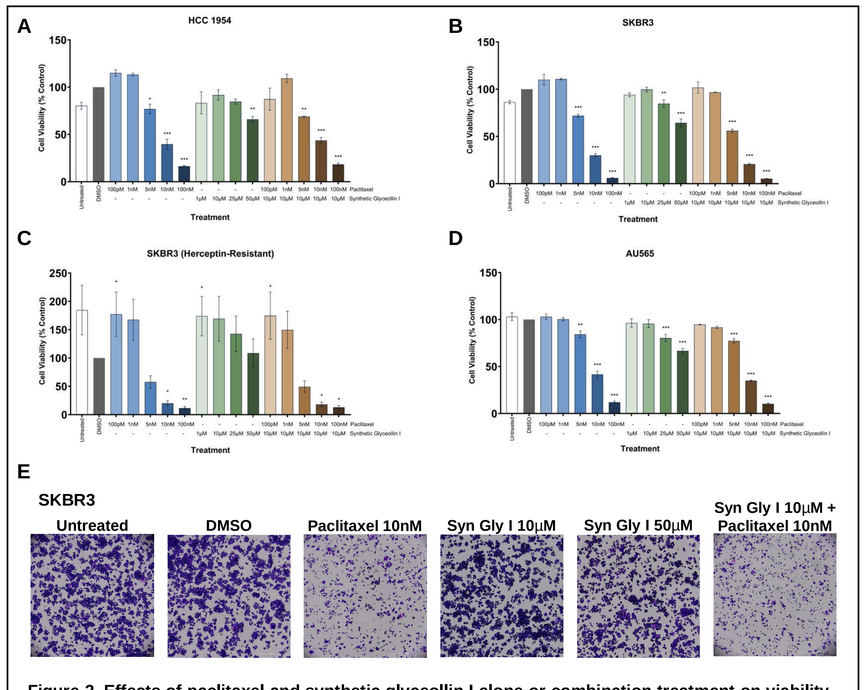


Figure 2. Effects of paclitaxel and synthetic glyceollin I alone or combination treatment on viability of HER+ cell lines. HER+ cell lines **(A)** HCC 1954, SKBR3 **(B)** parental and **(C)** herceptin-resistant, and **(D)** AU565 mCherry were plated on 96-well costar flat-bottom plates (6K cells per well for all cell lines, except AU565 at 10K cells per well). Cells were treated with vehicle (DMSO), paclitaxel, and synthetic glyceollin I (Syn Gly I) alone or in combination. On day 3 post treatment, plates were fixed and stained with crystal violet **(E** (SKBR3)**)**. Plates were imaged for cell morphology analysis using the BioTek Cytation 5 Imaging Reader (CY). Plates were eluted with acetic acid then read at 590nm on the CY. Data shown represent mean ± SEM, n=3. * p-value < 0.05; ** p < 0.01; *** p < 0.001.

Effects on colony formation

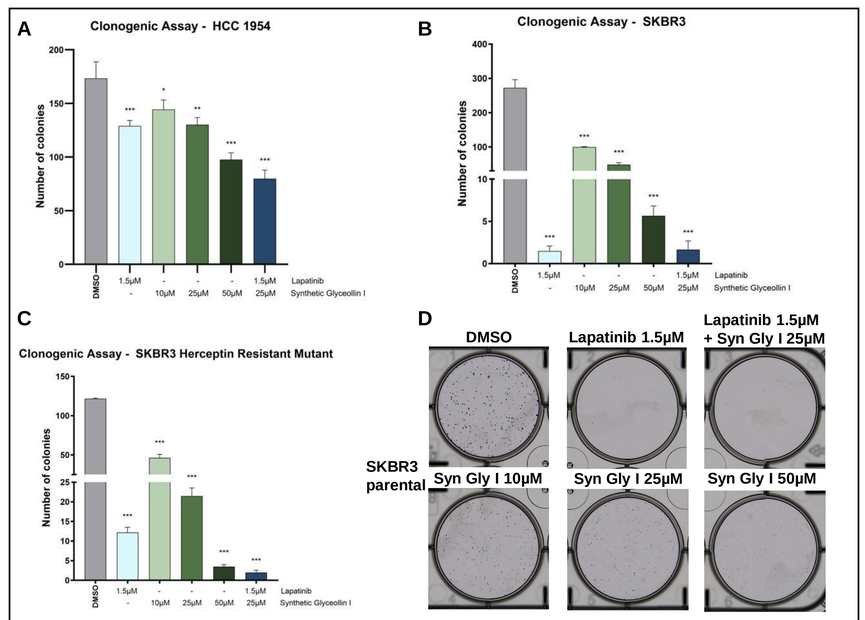


Figure 3. Synthetic glyceollin I inhibits colony formation of HER+ cells. HER+ cell lines **(A)** HCC 1954, **(B)** parental SKBR3, and **(C)** Herceptin-resistant SKBR3 were plated on 6-well flat bottom plates (1,000 cells per well) and treated with vehicle, lapatinib, or synthetic glyceollin I (10μM, 25μM, and 50μM) alone and in combination. Cells were cultured until colonies of 30-50 cells formed (at least one week), which varied between cell lines. Cells were fixed with glutaraldehyde then stained with crystal violet and imaged using the CY. Graphs were generated with GraphPad Prism software. Data shown represent average number of colonies ± SEM, n=3. * p-value < 0.05; ** p < 0.01; *** p < 0.001.

Differential Gene Expression

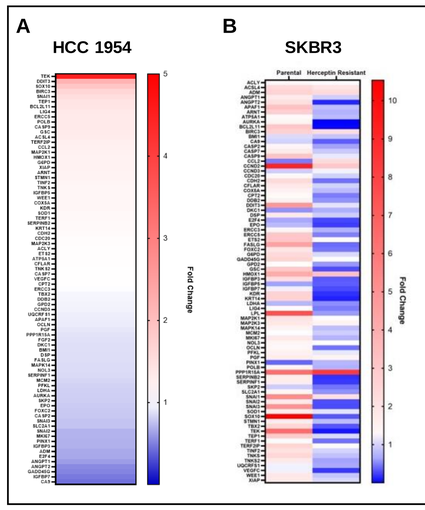


Figure 4. Cancer pathway analysis of cells treated with synthetic glyceollin I. HER+ cell lines **(A)** HCC 1954, **(B)** parental SKBR3, and herceptin resistant SKBR3 were treated with synthetic glyceollin I (25μM) for 24 hours. RNA was extracted from cell pellets using the Zymo Research Quick-RNA MiniPrep Kit RNA Extraction Kit. Concentration and purity of RNA samples were evaluated using a Nanodrop, and cDNA was synthesized via the Qiagen RT First Strand Kit cDNA Synthesis Kit. Gene expression was assessed using the Qiagen Human Cancer Pathway Finder PCR Array Plate. PCR array plates were run on the CFX Opus 96 RT-PCR System, and heat maps were generated using GraphPad Prism software.

Conclusions and Future Directions

Conclusions

- Synthetic glyceollin I treatment significantly inhibits proliferation and viability of HER+ breast cancer cells in a dose-dependent manner.
- Expression of cancer-related genes was altered in HCC1954 cells treated with Syn Gly I, notably TEK which is involved in angiogenesis.
- The majority of genes in the cancer pathway PCR array were downregulated in Syn Gly I-treated herceptin-resistant SKBR3 cells compared to herceptin-responsive cells, suggesting enhanced sensitivity of the former to Syn Gly I treatment.

Future Directions

- Assess effects of Syn Gly I treatment on cell viability and gene expression of additional herceptin-responsive and -resistant HER+ cell lines.
- Perform qRT-PCR to validate PCR array gene targets.

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