

## Disruption of Porphyrin Homeostasis by Inhibiting ABCG2 with Cyclohexylmethyl Flavonoids Suppresses Propagation of Stem-like Breast Cancer Cells

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Breast cancer stem cells express ATP-binding cassette sub-family G member 2 (ABCG2) and display multidrug resistance. Recent studies have shown that suppression of ABCG2 affects self-renewal of embryonic stem cells and inhibits proliferation of breast cancer cells. We therefore hypothesized that ABCG2 is involved in the maintenance of breast cancer stem cells and that cytotoxic ABCG2 inhibitors can be utilized to eliminate drug-resistant breast cancer stem cells. We have found that the ABCG2<sup>+</sup> subpopulation of MCF-7 cells is able to efflux protoporphyrin IX (PPIX), suggesting that the endogenous role of ABCG2 in breast cancer cells is to transport excess porphyrins. Utilizing a porphyrin-efflux assay, we have identified two cytotoxic cyclohexylmethyl flavonoids isolated from *Helminthostachys zeylanica* that can inhibit ABCG2 by binding to its nucleotide-binding domain. Treatment with ugonin J or K not only inhibited porphyrin efflux and the side-population phenotype, but induced apoptosis in both ABCG2<sup>+</sup> and ABCG2<sup>-</sup> cell populations and suppressed expansion of ABCG2<sup>+</sup>CD24<sup>-/low</sup>CD44<sup>+</sup> stem-like breast cancer cells in mammosphere cultures. We also found that the suppressive effect of ugonin J on propagation of stem-like breast cancer cells was mediated by PPIX accumulation, which in turn led to activation of p53 and reduction of Nanog. Overexpression of Nanog counteracted the suppressive effect of ugonin J. In conclusion, the current work identifies two novel cyclohexylmethyl flavonoids that can act as cytotoxic ABCG2 inhibitors to induce apoptosis in breast cancer cells and suppress propagation of stem-like breast cancer cells via reduction of Nanog.