In orthotopic xenografts and a PDX, our anticancer drug ErSO eradicates primary and metastatic therapy-resistant estrogen receptor alpha (ERα) positive breast cancer, and induces near-complete regression of ovarian cancer. The mechanism by which ErSO induces necrosis and kills ER positive cancer cells was unknown. From genome-wide CRISPR-Cas9 screens in MCF-7 and T47D cells with negative selection against first-generation BHPI and second-generation ErSO, and follow-on experiments, we identified the Ca2+ activated, plasma membrane Na+ channel TRPM4 as the executioner protein that BHPI and ErSO use to induce necrosis. Notably, in 6 ERα+ breast and ovarian cancer cell lines, knockout of TRPM4 completely abolished the ability of ErSO to induce death of cancer cells. Moreover, TRPM4 mRNA and protein were dramatically down-regulated in breast cancer cells selected for resistance to BHPI and ErSO. Furthermore, in a mouse xenograft, while ErSO induced near complete regression of orthotopic MCF-7-ERY537S-luciferase tumors, ErSO had no effect on the TRPM4 knockout tumors, which continued their robust growth. Since necrosis, but not most other death pathways, activates immune cells, inducing immunogenic cell death, this provides a new avenue for enhancing cancer immunotherapy. Importantly, medium from ErSO-treated wild type MCF-7 cells, but not medium from TRPM4 knockout cells, robustly activates human THP-1 monocytes and greatly increases their migration. BHPI and ErSO-induced initial anticipatory unfolded protein response (a-UPR) activation results in elevated cytosolic Ca2+, opening the plasma membrane TRPM4 channel, eliciting a rapid influx of external Na+, accompanying Cl- to balance the charge, and water to maintain osmolality. This swells the cells, causing osmotic stress. Importantly, it is the osmotic stress that sustains UPR hyperactivation, leading to ATP depletion, which contributes to membrane rupture and rapid necrotic cell death and to near complete inhibition of protein synthesis that ultimately kills any surviving cancer cells . Suggesting a broad role of TRPM4 in the actions of necrosis inducing anticancer drugs, TRPM4 knockout also inhibited necrosis induced by unrelated anticancer therapies, the mitochondrial targeting oncolytic peptide, LTX-315 and the Ca2+ channel targeting agent, Englerin A. Since increasing expression TRPM4 by viral transduction results in progressively increased sensitivity of ER positive breast cancer cells to killing by ErSO, this enables identification of breast cancer patients whose elevated TRPM4 levels make them most likely to benefit from this novel therapy. The TRPM4 pathway is a new mechanism for sustained lethal activation of the UPR and for targeting ER positive breast and ovarian cancer.

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How Strong and Sustained Activation of the Estrogen Receptor-mediated Anticipatory Unfolded Protein Response Kills Breast and Ovarian Cancer Cells

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