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## A routine method for the detection of endoplasmic reticulum stress in cancer cell lines

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구 ndoplasmic reticulum (ER) stress is defined as an imbalance between the ER's protein folding load and folding capacity, resulting Lin an accumulation of misfolded proteins in the ER lumen. ER stress can be induced by various physiological conditions, activating coordinated signal transduction pathways, collectively known as the unfolded protein response (UPR), to re-establish ER homeostasis. Uncontrolled cancer cell growth creates stressful conditions in the tumor microenvironment, inducing UPR activation and maintaining a state of chronic ER stress, which plays a vital role in tumor survival. The importance of the UPR in maintaining malignancy has encouraged research into the possible therapeutic possibilities of targeting the UPR and ER stress. Normal cells do not typically experience chronic ER stress; therefore, the controlled exacerbation of pre-existing ER stress in cancer cells may overload the system, resulting in cancer cell death, without affecting non-tumourigenic cells. Therapy-induced ER stress has been shown to target multiple hallmarks of cancer simultaneously, which is advantageous as current therapies are designed to target individual hallmarks. Despite the growing evidence of the importance of ER stress in cancer malignancy, there is no existing routine, high-throughput method to detect and quantify ER stress in cancer cell lines. This research optimized a routine method to detect and quantify ER stress in cancer cells using a non-conventional fluorescent dye, Thioflavin T (ThT). ThT binds selectively to protein aggregates, which are characteristic of ER stress. The results of this study indicate a substantial increase in fluorescence in the cell lines (MCF7, MDA-MB-231, MDA-MB-468) treated with ER stress-inducing compounds (brefeldin A, tunicamycin, thapsigargin), correlating with UPR activation. This correlation was evident across all ER stress-inducing treatments used. Therefore, to conclude, this method shows promise for the potential use as a routine, high-throughput method to detect and quantify ER stress.

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