

# Targeting of Epichaperome Downregulates HCFC1 Mediated Transcription of Oncogenes: Implications in Breast Cancer Therapy

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Breast cancer, annually affecting over 2.3 million people worldwide, is targeted in this study through the epichaperome, a network of proteins including heat shock protein 90 (HSP90). PUH71, a specific HSP90 inhibitor, disrupts cancer-specific epichaperome complexes, distinguishing between cancerous and healthy cells. PUTCO2, a novel PUH71 derivative, was previously developed using click chemistry, and was then used in this study for specific visualization of the entire epichaperome complex. Using PUTCO2-based confocal microscopy, the epichaperome was visualized in cell lines and identified as a biomarker for aggressive cancers such as MDA-MB-468 and MDA-MB-231 ( $p < 0.0001$  for both). A novel in-gel fluorescence assay, using PUTCO2, was developed to visualize changes in the epichaperome's expression. Western blots then quantified changes in epichaperome components, demonstrating that PUH71 decreases the expression of integral epichaperome-conformation HSP90 ( $p < 0.0001$ ). Semi-quantitative PCR and qPCR analyses revealed PUH71 reduced oncogenic expression of CDC42, Cyclin A, and BRCA1 ( $p < 0.0001$ ,  $p < 0.0001$ ,  $p = 0.0001$  respectively). This indicates epichaperome regulated oncogenic transcription, particularly in aggressive cancers like MDA-MB-468. CDC42 PCRs additionally suggest epichaperome involvement with the regulatory HCFC1 complex. Geldanamycin, a non-specific HSP90 inhibitor, showed lower specificity, confirming its ineffectiveness for breast cancer treatment. Future research should delve into molecular epichaperome mechanisms for gene control and PUTCO2's diagnostic potential. While this study is solely concentrated on breast cancer, the epichaperome remains a crucial and promising molecular target in a wide range of pathologies.