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Homoharringtonine Modulates Chemokine Expression through Phosphoglycerate Dehydrogenase Inhibition in Triple Negative Breast Cancer

Phosphoglycerate dehydrogenase (PHGDH) acts as the rate limiting enzyme for de novo serine biosynthesis. This characteristic has made PHGDH a promising new therapeutic target in cancers reliant on de novo serine synthesis for metastasis or in the nutrient-limited microenvironment of the brain. Certain 'moonlighting' functions in PHGDH have also been identified outside of its metabolic niche, such as the regulation of gene transcription and translation. The following study explores the potential of PHGDH inhibition in triple negative breast cancer (TNBC), a subtype characterized by its lack of estrogen, progesterone receptor, and human epidermal growth factor receptor-2 overexpression. The lack of targetable surface receptors, high metastatic potential, and extreme tumor heterogeneity make TNBC incredibly difficult to treat, and thus, there is an urgent need for novel targets to suppress tumor growth.

Compared to other breast cancer subtypes, PHGDH expression is significantly upregulated in TNBC. We hypothesize PHGDH inhibition may prove effective in targeting TNBC and expand possibilities for combinatorial therapy. Notably, preliminary data in PHGDH-knockdown TNBC tumors in mice show an increase in the chemokines CXCL9/10. In general, TNBC exhibits poor immune cell infiltration and thus responds poorly to traditional immunotherapies. Induced overexpression of chemokines within TNBC tumors may serve as a viable method for expanding possibilities for combinatorial immunotherapy. Furthermore, by targeting PHGDH with inhibitors with a clinical history, such as homoharringtonine (HHT), clinical translation may be accelerated.

HHT had been used previously as an anti-leukemia drug in its capacity as a protein synthesis inhibitor. Provided with the scope of this project, preliminary experiments sought to determine the dose by which PHGDH was inhibited, but protein synthesis was preserved. To assess dose-response and determine the sub-cytotoxic window, cell viability assays with PHGDH overexpressing (MDA 468, Hs578t, HCC 70, HCC 1937, MDA 436) and under expressing (BT 549, MDA 231, HCC 1806) human TNBC lines were conducted with HHT as well as known PHGDH-specific inhibitors (NCT 503, BI-4916, BI-4924). To confirm PHGDH inhibition and protein synthesis preservation in the sub-cytotoxic window, we ran western blots to assess PHGDH protein level and verify whether markers confirming protein synthesis inhibition (Cyclin D1) or G₁-M arrest (p27) were elevated upon treatment. Changes in chemokine expression were interrogated by qPCR. MTT assays demonstrated sensitivity to HHT in the nanomolar range for

all TNBC lines tested. Western blot data confirmed HHT at sub-cytotoxic doses decreased PHGDH content while preserving protein synthesis. Further, qPCR demonstrated upregulation of CXCL10 gene expression in all overexpressing lines treated with HHT (MDA 468, Hs578t, HCC 70, HCC 1937). Future cell-based assays will be conducted with more overexpressing lines treated with other known inhibitors to confirm PHGDH implication.

The severe lack of effective, targeted therapies to impair TNBC tumor progression calls for the investigation and exploitation of understudied axes. Preliminary data suggests that PHGDH inhibition may be a viable method to turn TNBC tumors from immune “cold” to “hot,” expanding opportunities for immunotherapy.