Inhibition of the Hexosamine Biosynthetic Pathway Impairs PIKK Maturation and Induces a BRCAness-Like Phenotype in Triple-Negative Breast Cancer

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Abstract:
Breast cancer (BC) is the most prevalent malignancy in women worldwide and remains a leading cause of cancer-related mortality. Therapeutic efficacy is particularly limited in Triple-Negative Breast Cancer (TNBC), which frequently develops resistance, relapse, and metastasis. Metabolic reprogramming in BC, including enhanced glycolysis and upregulation of the Hexosamine Biosynthetic Pathway (HBP), supports tumour growth and DNA damage response (DDR) via O-glycosylation of PIKK kinases (ATM, ATR, DNA-PK). We developed FR054, a selective inhibitor of PGM3, a key HBP enzyme, and evaluated its antitumor activity in BC cell lines. FR054 reduced proliferation and induced cell death both as monotherapy and in combination with the DNA-damaging agent Gemcitabine. Mechanistically, FR054 increased DNA damage, evidenced by elevated H2AX phosphorylation, and impaired activation of DDR kinases. Investigation of the RUVBL1/2 ATPase complex, an HSP90 co-chaperone required for PIKK maturation, suggested that HBP inhibition disrupts PIKK integrity. Based on these findings, we hypothesized that FR054 induces a “BRCAness-like” phenotype. Consistent with this, the combination of FR054 and the PARP inhibitor Olaparib markedly reduced cell proliferation at low doses and further increased DNA damage. In vivo validation was performed using a zebrafish xenograft TNBC model, where treatment with FR054 significantly impaired tumour cell expansion and migration compared to control and showed no overt signs of toxicity in zebrafish embryos, supporting its potential therapeutic window. These preliminary results indicate that targeting HBP with FR054 may sensitize TNBC cells to PARP inhibition by functionally impairing BRCA-related DDR pathways, offering a promising strategy for overcoming therapeutic resistance.