From genetic profiling to mechanistic insights: elucidating the functions of NFATC2IP and C16orf72 in genome maintenance and p53 regulation

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Doctor of Philosophy

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2025

Abstract

Adapting CRISPR/Cas9 technology as a forward genetic tool has enabled high-throughput, unbiased functional surveying of genes, therefore advanced elucidation of genome-scale genetic interaction landscapes. Cells heavily rely on post-translational modifications (PTMs) to drive context-specific protein interactions and accomplish dynamic regulation of signaling networks with a limited number of protein-coding genes. Ubiquitin (Ub) and ubiquitin-like protein (Ubl)-mediated PTMs are essential for protein homeostasis by bridging interactions between modified proteins and the proteasome, therefore are potential therapeutic targets. Although several Ub/Ubl-PTM pathway inhibitors are in clinical trials, their mechanisms of action and cellular pathways driving their therapeutic efficacies have not been described fully. Using CRISPR/Cas9 screens, I charted the chemogenetic architecture of cellular responses to clinical-stage Ub/Ubl-PTM pathway inhibitors. I uncovered a strong chemogenetic vulnerability of *NFATC2IP* with SUMOylation inhibition. NFATC2IP is an evolutionarily conserved protein with two SUMO-like domains that functions primarily in interphase to prevent defective chromosome segregation and genome instability when SUMOylation is inhibited. NFATC2IP promotes SUMOylation of

chromatin-associated proteins and interacts with the SMC5/6 complex and the SUMO E2 enzyme UBC9. Together with localization of NFATC2IP and SMC5 to nascent DNA, my work suggests its involvement in post-replicative resolution of DNA intermediates from replication or recombination. I propose that NFATC2IP interacts with UBC9 to stimulate the SUMO E3 ligase NSMCE2 within the SMC5/6 complex. Together, I conclude that NFATC2IP mediates SUMO-dependent genome maintenance in concert with the SMC5/6 complex.

Upon genotoxic stresses, cells utilize DNA repair pathways for survival and proper inheritance of genetic information, and p53 plays a key role in cell cycle arrest and apoptosis in response to DNA damage. p53 signaling is regulated by factors modulating p53 ubiquitylation, and viability upon loss of these factors is often determined by p53 status. Using CRISPR/Cas9 screens, I systematically chart such genetic interactions and uncovered a synthetic viability of *C16orf72/HAPSTR1/TAPR1* with *TP53* loss. I showed that C16orf72 acts as a negative regulator of p53 protein stability, likely through HUWE1 and USP7. The collaborative work revealed an accelerated tumour formation upon C16orf72 overexpression in a p53-dependent manner, highlighting potential impact of *C16orf72* and *USP7* co-gain/amplification in breast cancer progression.