Harnessing Saccharomyces cerevisiae to Assess a Novel Genetic Circuit Targeting p53 & Mdm2 for Cancer Therapy

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The tumor suppressor protein p53, is mutated in 50% of human cancers. DNA damage activates kinases which stabilize p53 through phosphorylation and paradoxically can promote cancer cell survival when p53 is mutated. Current p53-targeting therapies aim to deliver wild-type p53 (wt-p53) or inhibit its negative regulator Mdm2. However, they face two main challenges: (1) directing p53's (variable) downstream effects towards apoptosis and (2) overcoming dominant-negative (dn-p53) variants that inhibit wt-p53 activity. To address these limitations, I designed a novel cancer therapeutic approach for targeted protein expression termed Genetic Circuit Therapy (GC-Therapy), and tested it in a p53-responsive Saccharomyces cerevisiae strain. The system independently expresses p53 fusion proteins containing a fluorescent reporter protein (EGFP) or pro-apoptotic proteins (hBAX or iCasp9), as well as Mdm2 under two independent promoters. I assessed p53 activity using an ADE2-based colorimetric colony assay, and EGFP activity using fluorescence measurements. p53 and EGFP activity was reduced by increasing Mdm2 expression and restored upon repressing Mdm2. Neither pro-apoptotic protein (hBAX/iCasp9) was able to significantly reduce cellular growth. Higher p53 expression outcompeted Mdm2 inhibition, suggesting a threshold effect. Fusions containing EGFP all expressed fluorescence, indicating proper folding and successful p53-tetramerization. However, Mdm2 did not fully abolish p53-EGFP fluorescence, likely due to immediate EGFP activity before degradation. This suggests that therapies should consider p53-fusion partners that have delayed rather than immediate effect so Mdm2 has time to degrade the protein. My findings offer preliminary insights into engineering similar genetic-circuits.

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