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Identifying a novel HSP40/J-domain protein inhibitor that depletes mutant p53 to inhibit cancer malignancy

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Research Abstract Title

Identifying a novel HSP40/J-domain protein inhibitor that depletes mutant p53 to inhibit cancer malignancy

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IRB Number:

Describe role of Submitting/Presenting Trainee in this project (limit 150 words):
Submitting/presenting trainee (S.N) performed the experiments, processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures.

Background, Objectives/Goal, Methods/Design, Results, Conclusions limited to 500 words

Background:
Accumulation of oncogenic mutant p53 (mutp53) greatly contributes to cancer progression. DNAJA1, which is a member of heat shock protein 40 (HSP40), also known as J-domain proteins (JDPs), plays a crucial role in the stabilization of misfolded forms of mutp53. Knockdown of DNAJA1 results in proteasomal degradation of misfolded mutp53, leading to tumor suppression. Currently, no HSP40/JDPs inhibitors are available in clinics.

Objectives/Goal:

The goal of this study is to identify and characterize potential anti-cancer compounds which can induce mutp53 degradation by inhibiting HSP40/JDPs.

Methods/Design:
To identify compounds that potentially bind to DNAJA1, we performed an in-silico docking study for the J-domain of DNAJA1, whose NMR structure is available, using the ZINC database of commercially available compounds. Identified compounds and their analogs were validated for their abilities to deplete p53 and/or DNAJA1 in cancer cells with different p53 status by western blotting and immunofluorescence.

Results:
One of the top candidates, namely #7-3, effectively reduced the levels of DNAJA1 and misfolded mutp53. Also, an analog of #7-3, namely A#11, showed an even stronger activity to deplete both DNAJA1 and misfolded mutp53. Importantly, A#11 had minimal effects on the levels of wild-type p53 and DNA contact type of mutp53. Moreover, A#11 suppressed migration and filopodia formation in a mutp53-dependent manner. The docking study also predicted that three amino acids at tyrosine 7 (Y7), lysine 44 (K44), and glutamine 47 (Q47) in the J-domain are crucial for binding of A#11 to DNAJA1. Inserting missense mutations in these amino acids (Y7A, K44A, Q47A) attenuated the effects of A#11 on depleting DNAJA1 and mutp53 levels. Other HSP40/JDPs having Y7, K44 and Q47, including DNAJA2, DNAJA3, and DNAJB6 were also depleted by A#11. However, The response to A#11 was attenuated in HSP40/JDPs lacking one of these three amino acids, such as DNAJA4, DNAJB1, DNAJB2, DNAJB12, DNAJC3, and DNAJC7. Moreover, HSP40/JDPs lacking all of these three amino acids, such as DNAJC6 and DNAJC10 failed to respond to A#11.

Conclusions:
Our study has identified a small compound that inhibits DNAJA1 and other HSP40/JDPs, leading to depletion of misfolded mutp53 and reduced cancer malignancy.