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Novel HSP40/J-domain protein inhibitors to deplete misfolded mutant p53

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Research Abstract Title

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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. Heat shock protein 40 (HSP40), also known as J-domain proteins (JDPs), has been implicated in stabilization of misfolded forms of mutp53. Specifically, we demonstrate that DNAJA1, a member of HSP40/JDPs, binds to and stabilizes misfolded mutp53, while knockdown of DNAJA1 results in CHIP ubiquitin ligase-mediated degradation of misfolded mutp53 and inhibition of tumor growth. Since no HSP40/JDPs inhibitors are currently available in clinics, these findings prompted us to identify inhibitors for DNAJA1 or HSP40/JDPs.

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:
Our screening identified over 200 compounds as potential candidates. The top 33 compounds were tested for their abilities to reduce the levels of misfolded mutp53 and DNAJA1 by western blotting and immunofluorescence in KHOS/NP (p53R156P) and HN31 (p53C176F) cells, allowing us to identify the best candidate, namely #7-3. Eighteen (18) commercially available analogs of #7-3 were further examined for their potential to deplete mutp53 and DNAJA1. Of these, a compound, namely A#11, had the strongest activity to deplete both DNAJA1 and misfolded mutp53 (R156P, Y220C, C176F) in multiple cancer cell lines. A#11 reduced misfolded mutp53 in a concentration-dependent and a time-dependent manner without affecting mRNA levels of mutp53. Importantly, A#11 showed minimal effects on the levels of wild-type p53 and DNA contact mutp53 (R248L, R280K). Since there is a high homology in the J-domain of HDP40/JDPs, we next examined whether A#11 could also deplete other HSP40/JDPs. Indeed, A#11 reduced protein levels of other HSP40/JDPs, including DNAJA2, DNAJA3, DNAJA4, DNAJB1, DNAJB2, DNAJB6, DNAJC3, and DNAJC7; however, it had little impact on the levels and cellular localization of DNAJB12, DNAJC6, and DNAJC10.

Conclusions:
Our study, for the first time, has identified a small compound that inhibits or depletes multiple HSP40/JDPs, including DNAJA1, leading to reduced levels of misfolded mutp53. This compound and its analogs could be used to inhibit tumor progression as potential novel anti-cancer agents.