Mutant p53 degradation by potential HSP40/J-domain protein inhibitors derived from a natural compound plumbagin

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Describe role of Submitting/Presenting Trainee in this project:
Under the supervision of my Mentor, I participated in the conceptual design of the research project and conducted the research assays and contributed to the interpretation of data.

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

Background/Rationale: Accumulation of mutant p53 (mutp53) in cancer cells plays a crucial role in enhanced malignant properties by mutp53 oncogenic gain of function i(GOF). Given that mutp53 is required for cancer cells' abilities to maintain the oncogenicity, promoting mutp53 degradation is a promising new avenue for anticancer treatment. Recently, we identified a key role of DNAJA1, a member of J-domain proteins (JDPs/HSP40) family, in protecting mutp53, mainly the unfolded type, from degradation. Inhibiting the activity of DNAJA1 or JDPs may lead to reduced cancer progression through inducing mutp53 degradation; however, there is no JDPs/HSP40-specific inhibitors that are clinically available. To find DNAJA1 inhibitors, we performed in-silico docking studies and identified compounds derived from plumbagin, namely PLINH, which could potentially bind to the J-domain of DNAJA1. We hypothesized that PLINH analogs induce degradation of mutp53 through inhibition of DNAJA1's activity to stabilize misfolded mutp53.

Objectives/Goal: Our goal is to identify novel anti-neoplastic compounds derived from natural products that could induce specific degradation of misfolded mutp53.

Methods/Design: To verify the potential binding of PLINH to DNAJA1 protein in cells, we performed CETSA assays. Five PLINH analogs were then screened for their effects on promoting degradation of misfolded mutp53 (R156P, R175H, C176F), DNA contact mutp53 (R248L, R273H, R280K), and wild-type p53 (wtp53), using multiple cell lines with various p53 statuses, with and without DNAJA1 or mutp53 knockdown/knockout. MTT assays were performed to determine the toxicity and IC50 values of PLINH analogs using both cancer and non-transformed cells. The effects of these compounds on the level of

p53 and DNAJA1 proteins were assayed by immunoblotting and immunofluorescence. The biological effects of these compounds on inhibiting the malignant properties of cancer cells was measured by trans-well migration and filopodia formation assays.

Results: PLINH specifically decreased the levels of misfolded mutp53 with minimal or no effects on wtp53 and DNA contact mutp53. Three PLINH analogs including PLINH and PLTFBH showed similar efficacy on reducing the misfolded mutp53 levels with comparable cytotoxicity on cancer cells while having minimal effects on non-tumor cells. Our data indicate that the effects of PLINH analogs on cancer cells' viability and proliferation were not entirely dependent on DNAJA1 and misfolded mutp53, since these compounds were still toxic to the cells lacking DNAJA1 or misfolded mutp53. However, the inhibitory effects of PLTFBH on the migration and filopodia formation of cancer cells harboring misfolded mutp53 were specific, since the inhibitory effects were minimum in cells lacking DNAJA1 and mutp53. Our findings highlight the crucial role of a JDP member, DNAJA1, in promoting mutp53-mediated cancer migration and demonstrate the specificity of PLINH analogs in the inhibition of the DNAJA1/mutp53-mediated cancer cell migration.

Conclusions: PLINH analogs specifically inhibited migration and filopodia formation in cancer cells in a manner dependent on DNAJA1 and misfolded mutp53.

Significance: Identifying natural compounds that inhibit DNAJA1 activity to induce degradation of misfolded mutp53 could pave the way toward discovery of a promising targeted therapy for various cancer conditions.