Research Days

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Novel potential HSP40 inhibitors derived from a natural compound plumbagin effectively deplete mutant p53

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X Resident/Ph.D./postgraduate (> 1 month of dedicated research time)

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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, 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 facilitates tumor progression. A member of J-domain proteins family (JDPs/HSP40), namely DNAJA1 was recently found to promote stabilization of unfolded mutp53 through preventing its degradation.

Objectives/Goal: Cancer cells are addicted to mutp53, therefore, targeting DNAJA1 or other members of JDP protein family could lead to inhibition of cancer progression via the depletion of mutp53. Thus, our goal is to investigate potential DNAJA1 inhibitors which could provide a promising approach for cancer therapy.

Methods/Design: *In-silico* molecular docking analysis was performed using a library of molecules derived from natural compounds. To verify the binding of DNAJA1 protein to candidate small molecules in cells, CETSA assays were performed. The molecules were, thence, screened for their effects on the degradation of different forms of mutp53. Toward, this goal, cells harboring unfolded mutp53 (R156P, R175H, C176F), DNA contact mutp53 (R248L, R273H, R280K), and wild-type p53 (wtp53) were utilized. To verify the dependence on DNAJA1 and unfolded mutp53, the molecules were tested on an array of cell lines in the absence and presence of DNAJA1 or mutp53. MTT assays were performed to determine the toxicity and IC50 values of the tested compounds using several cancer and non-transformed cells. The effects of compounds on the level of p53 and DNAJA1 proteins were assayed by immunoblotting and immunofluorescence. The biological effects of the compounds on cancer cells' progression cells were evaluated using trans-well migration and filopodia formation assays.

Results: The docking analysis identified a plumbagin derivative, PLIHZ, that could potentially bind to J domain of DNAJA1 protein at tyrosine 8 residue (Y8). PLIHZ efficiently reduced the levels of several unfolded mutp53, but it exerted minimal effect on DNA contact mutp53 and wild-type p53 (wtp53). Three PLIHZ analogs were comparable to PLIHZ in terms of reducing DNAJA1 and

unfolded mutp53. An analog, called PLTFBH, specifically inhibited the migration of cancer cells carrying unfolded mutp53, while it exhibited minimal effect on the migration of cancer cells harboring DNA contact mutp53, p53-null, and wtp53. Moreover, the inhibitory effect of PLTFB on the migration of unfolded mutp53-bearing cancer cells was attenuated by the depletion of DNAJA1 or mutp53. Furthermore, PLTFBH reduced the levels of several Y8-containing members of JDPs/HSP40 protein family. Notably, substitution of Y8 tyrosine with alanine, in mutant DNAJA1 (DNAJA1Y8A), significantly decreased the ability of PLTFBH to bind to and deplete mutant DNAJA1. Together, these results strongly suggest PLTFBH as a potential inhibitor of a certain group of JDPs/HSP40 members.

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

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