May 11th, 11:30 AM - 1:30 PM

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

Mohamed A.A. Alalem

*Children's Mercy Hospital*

Follow this and additional works at: [https://scholarlyexchange.childrensmercy.org/researchdays](https://scholarlyexchange.childrensmercy.org/researchdays)

Part of the [Higher Education and Teaching Commons](https://scholarlyexchange.childrensmercy.org/researchdays), [Medical Education Commons](https://scholarlyexchange.childrensmercy.org/researchdays), [Pediatrics Commons](https://scholarlyexchange.childrensmercy.org/researchdays), and the [Science and Mathematics Education Commons](https://scholarlyexchange.childrensmercy.org/researchdays)


This Poster Presentation is brought to you for free and open access by the Conferences and Events at SHARE @ Children's Mercy. It has been accepted for inclusion in Research Days by an authorized administrator of SHARE @ Children's Mercy. For more information, please contact [library@cmh.edu](mailto:library@cmh.edu).
Mutant p53 degradation by potential HSP40/J-domain protein inhibitors derived from a natural compound plumbagin

Mohamed Alalem¹, Atul Ranjan², Satomi Yamamoto², Atsushi Kaida², Alejandro Parrales², Sana Farooki¹, Subhash Padhye³, Shrikant Anant², Tomoo Iwakuma¹,²

¹Children’s Mercy Kansas City, ²University of Kansas Medical Center, ³University of Pune

Abstract

Background/Rationale: Accumulation of mutant p53 (mutp53) in cancer cells plays a crucial role in enhanced malignant properties by mutp53 oncogenic gain of function (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 (wt p53), 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 wt p53 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.
Figure 1: The heat shock protein 40 member, DNAJA1, specifically protects conformational mutp53 from degradation.
Figure 2: PLIHZ reduces the level of DNAJA1 protein in all cancer cells, but it only reduces the level of conformational mutp53 in cancer cells.

(a) Cal33 (R175H)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temp. (°C)</th>
<th>DNAJA1</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unheated</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>40</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>43</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>46</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>49</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>52</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>55</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>58</td>
<td>D</td>
<td>P</td>
<td>D</td>
</tr>
</tbody>
</table>

Relative DNAJA1 (% control) vs. Temperature (°C)

(b) HN31 (C176F), HT29 (R273H), U2OS (p53wt), H1299 (p53null)

Compound:DMSO/PLIHZ

Scale bar 20µm
Figure 3: Identification of plumbagin derivatives that bind DNAJA1 protein and reduce the levels of conformational mutp53 in cancer cells.
Figure 4: PLTFBH compound inhibits cancer cells migration in a DNAJA1-mutp53-dependent manner.
Figure 5: PLIHZ analogs inhibit filopodia formation in cancer cells in a DNAJA1-mutp53-dependent manner.
Figure 6: PLTFBH compound decreases the level of several JDP/HSP40 proteins with lesser effect on DNAJC6 that lacks tyrosine 8.
1. PLINH compounds can potentially bind J-domain of HSP40 family members.

2. DNAJA1 protein contributes to the protection of conformational mutp53 from degradation.

3. PLINH induces degradation of the conformational mutp53 specifically while it has minimal effect on the WTP53 and DNA-contact counterparts.

4. PLINH compounds inhibit the migration potential of cancer cells harboring conformational mutp53 in a DNAJA1-mutp53 dependent manner.
Supplementary figure 1: DNAJA1 protects conformational mutp53 but not DNA-contact and wtp53.
Supplementary figure 2: The prototype plumbagin-derived compound, PLIHZ, binds to DNAJA1 and reduces the level of conformational mutp53.

(a) 24h-IC50 values for various cancer cells treated with PLIHZ.
(b) Western blot analysis of DNAJA1 and GAPDH expression after treatment with DMSO or PLIHZ.
(c) Immunofluorescence images showing DNAJA1 and DAPI staining in different cancer cell lines treated with DMSO or PLIHZ.
(d) MTT assay results showing cell viability after treatment with PLIHZ and DNAJA1 or p53 sgRNA.

p53 status:
- Unfolded DNA contact
- WT: cancer
- Null
- WT: non-tumor

Supplementary figure 3: Characterization of PLTFBH compound by the screening of five PLIHZ analogs for their efficacy and safety.

a) Bar graph showing 24h-IC50 values for PLTFBH in different cancer cell lines.

b) Western blot images for p53 and GAPDH expression under different conditions.

c) Heat map showing relative gene expression for DNAJA1 and TP53.

d) MTT assay results for HN31, CAL33, and KHOS/NP cells over 72h.
Supplementary figure 4: PLTFBH inhibits the migration of cancer cells in a DNAJA1-mutp53–dependent manner
Supplementary figure 5: PLTFBH compound inhibits filopodia formation specifically in cancer cells with unfolded mutp53.

(a)