Mutant p53 Depletion by Natural Compounds

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Mutant p53 depletion by Natural Compounds

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Abstract

**Background:** P53 protein is a major tumor suppressor protein that protects cells from becoming cancerous via the induction of cell cycle arrest or apoptosis. Approximately 50% of human cancers carry mutations in their P53 genes and the majority of these mutations are missense in nature. Mutated P53 protein is a well-known driver of cancer progression through the loss of tumor suppressor capacity of wild type p53 as well as gain of new oncogenic functions mediated by mutated P53. Therefore, targeting mutant P53 (mutP53) is a promising approach for treatment of various types of cancer.

**Objectives/Goal:** The goal of this research project is to identify and characterize potentially novel antineoplastic agents that could specifically degrade conformational mutP53 protein in cancer cells.

**Methods/Design:** Previous research in our lab has identified heat shock protein 40 (HSP40) to be implicated in protecting misfolded mutp53 from degradation by CHIP proteolysis. Docking studies revealed that natural compounds derived from curcumin and plumbagin can potentially bind to the J-domain of HSP40. Various human cancer cell lines were treated with curcumin derivatives and analogs of PLINH which is made by combination of plumbagin and isoniazid. These compounds exerted a significant inhibitory effect on the viability/proliferation of human cancer cells as shown by MTT assay. Results also indicate that the effect of these compounds involves DNAJA1-mutp53 axis. Immunoblotting assay ravelled that these compounds can induce a concomitant decrease in conformational mutP53 specifically in cells harboring conformational mutp53.

**Results:** Treatment of cancer cell lines with curcumin or PLINH showed that these compounds inhibit the proliferation and viability of several human cancer cells. Inhibition of cancer cells proliferation was associated with decreased level of mutP53 protein in these cancer cells. Moreover, our data indicate that the effect of these compounds on cancer cells is mediated through specifically targeting cells that harbor conformational mutP53 proteins.

**Conclusions:** Treatment of cancer cells with curcumin or PLINH compounds inhibit proliferation of cancer cells in a DNAJA1-misfolded mutp5-dependent manner.

**Significance:** Delineation of the exact mechanism through which curcumin and PLINH exert their anti-cancer effects could pave the way for promising novel preventive and therapeutic modalities for cancer.
Previous research in our lab identified a role of the HSP40, DNAJA1, in promoting stabilization of conformational mutp53. Docking studies showed that curcumin and PLINH can bind to the J-domain of DNAJA1 protein. Thus, our hypothesis is that curcumin and PLINH treatment could abolish DNAJA1- conformational mutp53 binding. This leads to an increased degradation of mutp53 and consequently inhibition of cancer growth and progression. Therefore, these compounds could be potentially exploited as potential preventative and adjuvant or neoadjuvant therapeutic modalities for cancers harboring misfolded mutp53 protein.
Curcumin and PLINH can potentially bind to J-domain HSP40 (DNAJA1) with high affinity

Docking Results in j-domain of DNAJA1

(2L01): (https://www.rcsb.org/structure/2l01)

<table>
<thead>
<tr>
<th>Name</th>
<th>B.E.(Kcal)</th>
<th>H bonds</th>
<th>Amino acids</th>
<th>Distance(Å)</th>
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</thead>
<tbody>
<tr>
<td>CDF</td>
<td>-6.4</td>
<td>1</td>
<td>LYS24-1</td>
<td>2.1</td>
</tr>
<tr>
<td>PIP-SF5</td>
<td>-6.8</td>
<td>1</td>
<td>SER50-1</td>
<td>2.4</td>
</tr>
<tr>
<td>PLINH</td>
<td>-6.6</td>
<td>1</td>
<td>TYR8-1</td>
<td>2.6</td>
</tr>
<tr>
<td>PLSA</td>
<td>-6.6</td>
<td>4</td>
<td>GLY43-1, LYS47-1, TYR27-1</td>
<td>2.4, 2.3 &amp; 2.7</td>
</tr>
<tr>
<td>PLBE</td>
<td>7.0</td>
<td>3</td>
<td>SER50-1, TYR27-1</td>
<td>2.2, 2.3 &amp; 3.2</td>
</tr>
</tbody>
</table>

Observations: Among the compounds screened, PLBE showed the highest binding affinity to DNAJA1.

Pip-SF5 (Analog of CDF) also showed good binding affinity to the protein and significantly better than CDF.

Overall, Plumbagin analogs showed better affinity.
Curcumin derivatives inhibit cancer cells viability through a DNAJA1-mutp53-dependent mechanism.
PLINH compounds also target the conformational mutp53 more preferentially than its DNA-contact counterparts.

<table>
<thead>
<tr>
<th>40uM/24hr</th>
<th>KHOS (R156P) conformational mutp53</th>
<th>HN31 (C176F) Conformational mutp53</th>
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<tbody>
<tr>
<td></td>
<td>DMSO  PLINH  FBH  TFBH  FUH  OCT</td>
<td>DMSO  PLINH  FBH  TFBH  FUH  OCT</td>
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</table>

<table>
<thead>
<tr>
<th>40uM/24hr</th>
<th>Panc-1 (R273H) DNA-contact mutp53</th>
<th>Mia-Paca-2 (R248W) Conformational mutp53</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMSO  PLINH  FBH  TFBH  FUH  OCT</td>
<td>DMSO  PLINH  FBH  TFBH  FUH  OCT</td>
</tr>
</tbody>
</table>
PLINH compounds have slight or no effect on wild type p53 or DNA-contact mutp53.
PLINH compounds decrease the level of DNAJA1 protein concomitantly with the decrease in mutp53

<table>
<thead>
<tr>
<th>Conformational mutp53</th>
<th>KHOS (R156P)</th>
<th>HN31 (C176F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40uM/24hr</td>
<td>DMSO</td>
<td>PLINH</td>
</tr>
</tbody>
</table>
Conclusion

1. Curcumin and PLINH compounds can potentially bind to the J-domain of HSP40 family members.

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

3. Curcumin and PLINH induce degradation of the conformational mutp53 more preferentially compared to the DNA-contact counterparts.

4. Both Curcumin and PLINH inhibit the viability/ proliferation of cancer cells harboring conformational mutp53 in a DNAJA1-mutp53 dependent manner.
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