Activation of PTEN lipid phosphatase function in lung cancer

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Aim:

To define and characterize novel peptidomimetics that directly activate PTEN function *in vitro* and abrogate oncogenic potential of lung cancer cells.

Rationale

Loss of PTEN activity in lung cancer induces oncogenic PI3K/AKT/S6K kinase cascade signaling. Therapy with kinase inhibitors (KIs) cause off-target effects and chemo-resistance, warranting the need for alternative therapy. PTEN activation represents a novel alternative therapeutic paradigm to mitigate PI3K/AKT/S6K signaling in cancers.

Hypothesis:

Direct activation of PTEN function will attenuate PI3K/AKT/S6K signaling, providing a novel entrée to treat lung cancers.

Findings:

1. *In vitro* screening of compounds using PTEN-phosphatase microtiter plate assays revealed select peptidomimetics that enhanced PTEN lipid phosphatase activity.
2. Select peptidomimetics activating PTEN, reduced oncogenic PI3K/AKT/S6K signaling, decreased cell proliferation, migration, and caused cell cycle arrest of lung cancer cells.
3. Molecular docking revealed a specific functional group within the peptidomimetics that plausibly enhance PTEN activity.

Clinical significance:

Multiple cancers and "PTEN-Opathies" (Harmatoma Syndromes, Autism, Alzheimer's disease, Parkinsonism and metabolic disorders/obesity), are associated with aberrant PTEN function. Emerging evidence from our lab and others suggest that conformational mediated PTEN inactivation directly contributes to PI3K/AKT/S6K oncogenic signaling in cancers, including lung cancer. Since KIs have limited success, we have used a novel approach to activate PTEN via peptidomimetics *in vivo*. Our strategy has the potential to attenuate disease phenotypes associated with reduced PTEN function, including lung cancer.

Exploiting PTEN structure for targeting with peptidomimetics

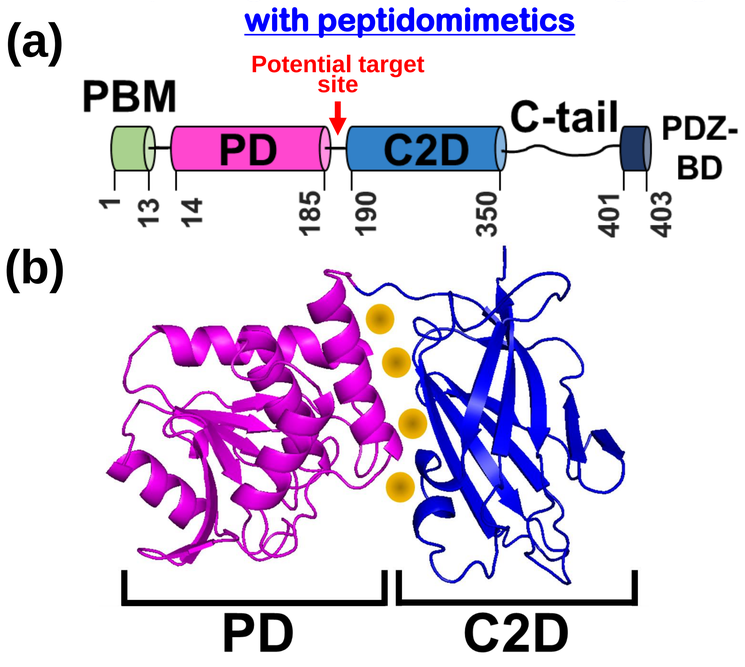

Fig. 1: Overview of PTEN structure. **(a)** PTEN has four domains: 1) a PIP_2 Binding Module at the N-terminus, 2) a catalytic Phosphatase Domain, 3) a C2 Domain and 4) a flexible C-tail harboring a PDZ-Binding Domain at the C-terminus. **(b)** PTEN crystal structure, targetable areas are marked with yellow sphere.

Synthesis of peptidomimetics

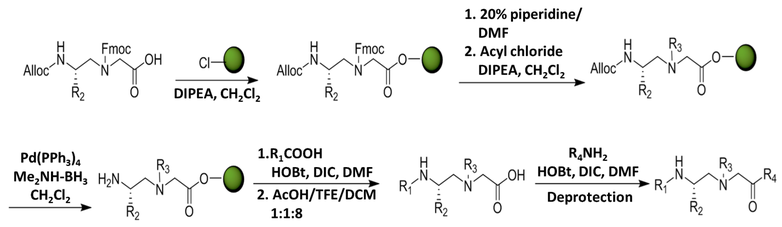

Fig. 2: Schematic of peptidomimetic synthesis. Bioactive peptides were synthesized from an N-acylated-N-aminoethyl backbone containing the R_2 side chain. R_1, R_3, and R_4 side chains were individually added to the backbone sequentially, following protection and deprotection steps.

Peptidomimetics activate PTEN lipid phosphatase function

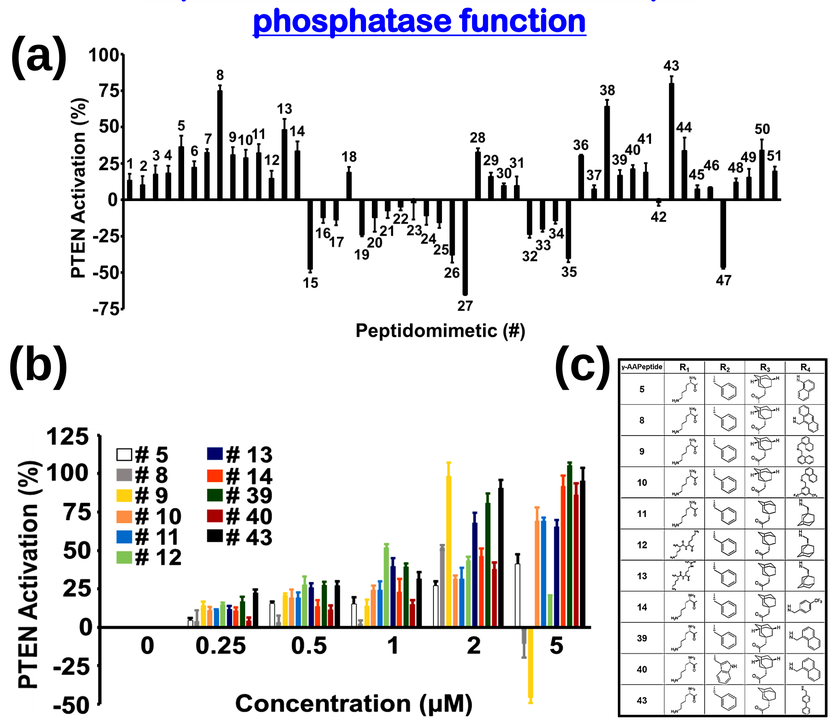

Fig. 3: Select peptidomimetics enhance PTEN activity *in vitro*. **(a)** Peptidomimetics were screened for changes in PTEN lipid-phosphatase activity at 1uM, using the Malachite Green phosphatase assay, n=4. **(b)** Select peptidomimetics enhanced PTEN activity in a dose-dependent manner, n=4. **(c)** Table of side chains for select peptidomimetics.

Select peptidomimetics inhibit PI3K/AKT/S6K pathway activation

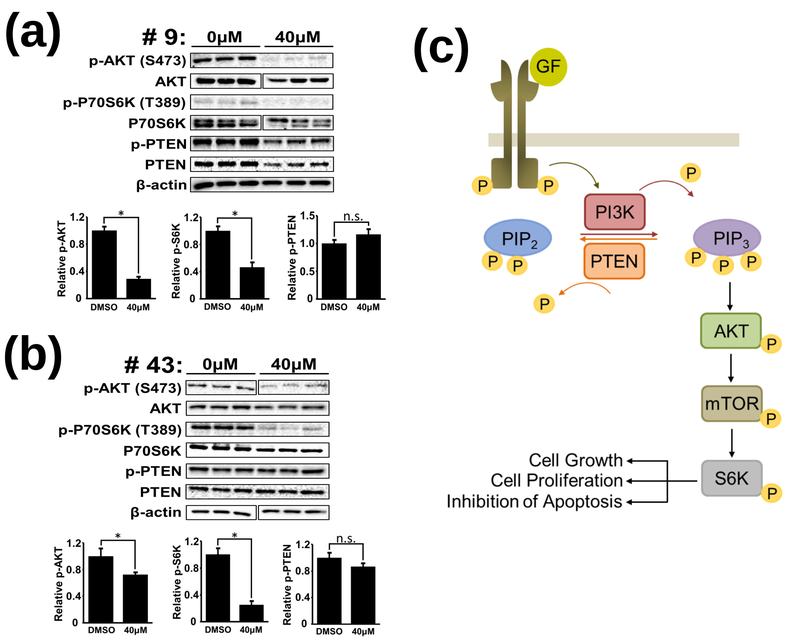

Fig. 4: Select peptidomimetics reduced PI3K/AKT/S6K pathway activation in lung cancer cells. **(a and b)** Peptidomimetic #9 and #43 reduced the phosphorylation of activated kinases p-AKT (Ser473) and p-P70S6K (Thr389), n=3 (*p value ≤0.05). **(c)** Role of PTEN in the PI3K/AKT/S6K pathway.

Select peptidomimetics decrease cell proliferation and migration

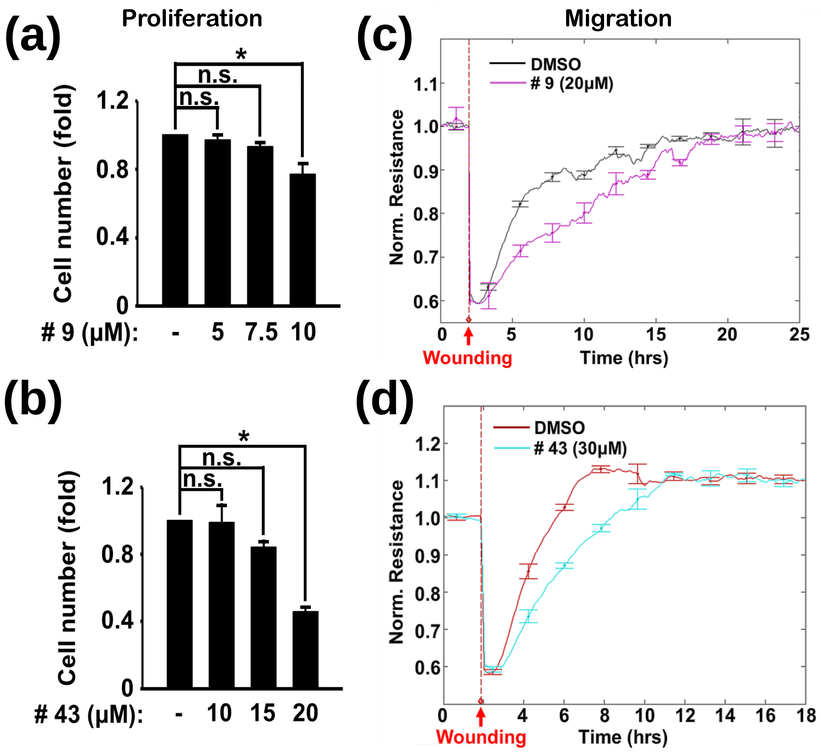

Fig. 5: Select peptidomimetics inhibit cell proliferation and migration in lung cancer cells. **(a and b)** Treatment with peptidomimetic #9 and #43 showed dose-dependent inhibition in cell proliferation. A549 cells plated at 9000 cells/well, 24 hour treatment, n=6 (*p value ≤0.05). **(c and d)** Peptidomimetic #9 and #43 reduced the rate of cell migration as measured by the ECIS method (treatments were 12 hours prior to wounding, n=3).

Select peptidomimetics induce cell cycle arrest

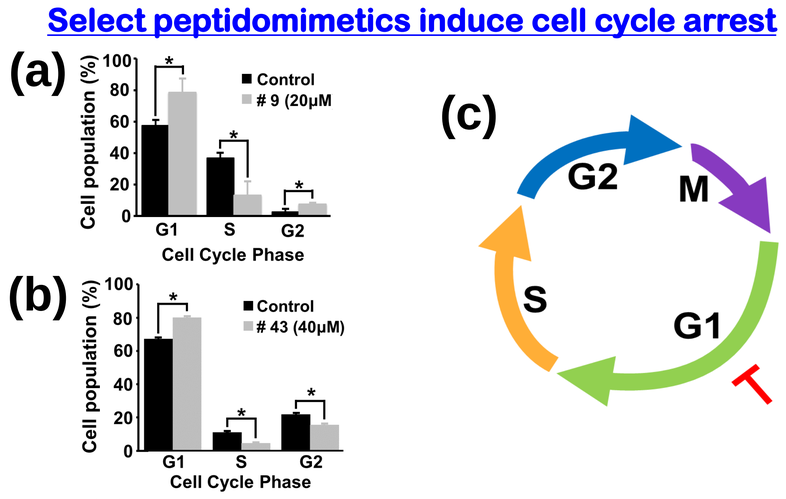

Fig. 6: Select peptidomimetics induced cell cycle arrest in lung cancer cells. **(a and b)** Treatment with peptidomimetic #9 or #43 induced cell cycle arrest at G1 phase, n=3 (*p value ≤0.05). **(c)** Cell cycle schematic.

FTMap analysis: Binding hotspots on PTEN protein

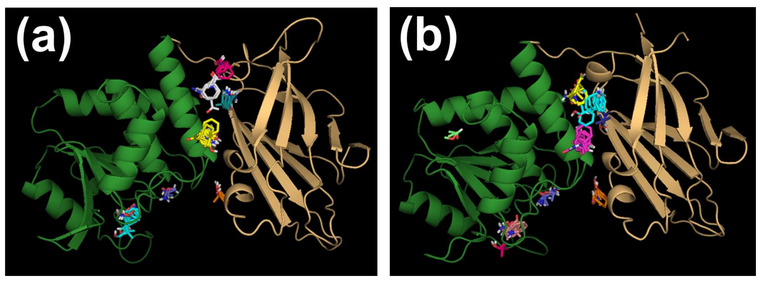

Fig. 7: FTMap analysis revealed binding hotspots on PTEN crystal structures. **(a)** The 16 standard probes aggregated near the alpha helical interface of the PD/C2D and near the CBR3 loop of PTEN (PDB 5BZZ). **(b)** The 16 standard probes aggregated near the alpha helical interface of the PD/C2D and near the CBR3 loop of PTEN (PDB 1D5R). Visualized in PyMol.

Molecular docking predicts low energy interactions of select peptidomimetics with PTEN protein

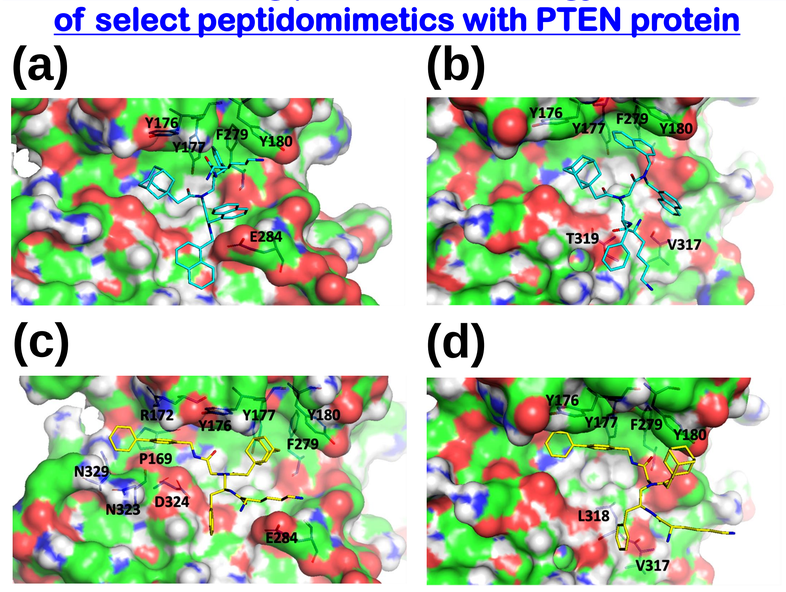

Fig. 8: Molecular docking revealed low energy interactions of peptidomimetics with PTEN crystal structures. **(a and c)** Peptidomimetic #9 and #43 docked to PTEN (PDB 5BZZ). **(b and d)** Peptidomimetic #9 and #43 docked to PTEN (PDB 1D5R). Peptidomimetic docking was directed to amino acid residues 170-185 at the PD/C2D interface, and residues 278, 279 near the CBR3 loop. Lowest energy interactions were visualized in PyMol.

Conclusions and clinical significance:

- Our peptidomimetics enhanced PTEN lipid phosphatase activity, antagonized PI3K/AKT/S6K signaling and attenuated the oncogenic potential of lung cancer cells.
- Molecular docking studies revealed that the adamantyl side chain in select peptidomimetics contributed to the interaction with PTEN protein, likely activating PTEN.
- Peptidomimetics represent an alternative therapeutic strategy for patients with diseases associated with hyper-activated PI3K/AKT/S6K signaling, or compromised PTEN function.

Future work:

- Co-crystallize select peptidomimetics with PTEN protein to elucidate definitive interactions.
- Develop an *in vivo* PDX mouse model to assess the role of select peptidomimetics in cancer progression.

References:

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