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TITLE
Exploring the Target Pathway of [D-Trp]CJ-15,208 in Prostate Cancer

HYPOTHESIS:
We expect that the anti-proliferative activity of our lead peptide [D-Trp]CJ-15,208 in c-Myc-overexpressing prostate cancer cell lines involves proteins in the c-Myc degradation pathway, and thus changes in the phosphorylation and expression of these proteins will be observed upon treatment with peptide. Furthermore, we expect that this activity will be maintained upon conservative modifications of the peptide structure during the development of probes to be used in target identification studies.

BACKGROUND/AIMS:
The oncoprotein c-Myc regulates a variety of genes, including those involved in cell growth, proliferation, and apoptosis. Overexpression of c-Myc is found in a variety of cancers, including breast and prostate cancer, and contributes to increased growth and cell survival. Thus, c-Myc is a promising target for the development of anti-cancer therapeutics. We have demonstrated that the macrocyclic tetrapeptide [D-Trp]CJ-15,208 has anti-proliferative activity in c-Myc-overexpressing prostate cancer cells, showing promise as a potential anti-cancer therapeutic. Although data suggests a role for the c-Myc degradation pathway in the activity of [D-Trp]CJ-15,208, the mechanism by which this peptide decreases proliferation remains unclear. Thus, our aim is to identify the molecular target of [D-Trp]CJ-15,208. The effects of this peptide on proteins involved in the c-Myc degradation pathway are being evaluated, and analogs are being developed to serve as probes in target identification studies.

METHODS:
The effects of [D-Trp]CJ-15,208 on the expression of proteins involved in the c-Myc degradation pathway were evaluated in PC-3 prostate cancer cells using western blot analysis. Additionally, a derivative of [D-Trp]CJ-15,208 was synthesized as a starting point for the development of additional probes, and its anti-proliferative activity was determined in the PC-3 prostate cancer cell line using the WST-1 assay.

RESULTS & CONCLUSIONS
We have demonstrated that [D-Trp]CJ-15,208 decreases c-Myc protein levels. This decrease was accompanied by a reduction in the inhibitory phosphorylation of protein phosphatase 2A (PP2A), a protein that dephosphorylates c-Myc to promote its degradation. This data suggests that the activity of [D-Trp]CJ-15,208 may involve increased degradation of c-Myc through activation of PP2A. However, it does not confirm the molecular target of the peptide. Thus, we have also begun synthesizing analogs that will be used as probes in pulldown studies to identify the molecular target. Our current [D-Trp]CJ-15,208 analog retained activity in a preliminary proliferation assay, suggesting that the modification introduced is tolerated and therefore suitable for the development of probes for future studies. Ultimately, by using these probes to identify the target and by understanding the pathways affected by [D-Trp]CJ-15,208, we will be able to develop promising leads for the treatment of c-Myc-overexpressing cancers.