FraAP, a Peptide Antagonist Against the Activator Protein 1 Transcription Factor Complex, Demonstrates Cancer Cell Cytotoxicity and Reduced Invasion In Vitro and Tumor Regression In Vivo in HNSCC Models

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SAPIENCE THERAPEUTICS

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Abstract

The AP-1 transcription factor complex, comprised of Fra1 and Jun heterodimers, plays a pivotal role in the pathogenesis of head and neck squamous cell carcinoma (HNSCC). This complex significantly contributes to tumor progression and metastasis with its expression positively correlating with poor prognosis. As dimerization of components of the AP-1 complex is required for DNA binding and subsequent transcriptional activity, we designed a peptide (Fra1 antagonizing peptide, FraAP) targeting the basic leucine zipper motif of AP-1 to antagonize AP-1 complex formation and prevent associated activity. Biolayer interferometry (BLI) and DNA ELISA assays demonstrate target binding and selectivity of FraAP towards AP-1 family members, co-immunoprecipitation experiments reveal that FraAP blocks protein-protein interactions between cJun and Fra1, and reporter assays confirm inhibition of AP-1 transcriptional activity in vitro. Transcriptomic analysis reveals that FraAP suppresses AP-1-driven pathways required for cell cycle progression, proliferation, and invasion, and promotes apoptosis. RNAseq data was confirmed by qPCR analysis of relevant target genes and functional in vitro assays. Annexin V characterization of FraAP treated cells by flow cytometry confirms that FraAP induces dose-dependent apoptosis. Cell cycle analysis confirms that FraAP induces G1 cell cycle arrest. FraAP exhibits an inhibitory effect on invasion in Boyden chamber assays and induces a phenotypic mesenchymal to epithelial transition characterized by decreased mesenchymal marker N-cadherin and increased epithelial marker E-cadherin. Further, in a HNSCC subcutaneous xenograft model, administration of FraAP results in significant tumor growth inhibition, demonstrating the anti-cancer potential of targeting the AP-1 complex. In summary, these data support FraAP as a potent peptide antagonist of the AP-1 transcription factor family that warrants further development as a potential therapeutic option for AP-1 driven tumors.

Targeting Activator Protein 1 (AP-1)

The cancer dependency map (DepMap portal), consisting of a genome-scale CRISPR-Cas9 essentiality screen across 342 cancer cell lines, identifies genes essential for proliferation and survival of cancer cells. Of the potential JUN/FOS heterodimeric interactions, the DepMap portal implicates the cJUN and FOSL1 (gene encoding for Fra1) interaction as crucial in cancer cell survival. We have designed a peptide antagonist, FraAP, targeting the basic leucine zipper motif of AP-1 to disrupt the interaction between cJUN and Fra1 and antagonize AP-1 activity.

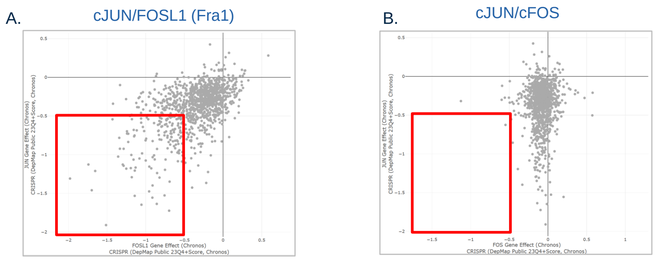


Figure 1. DepMap portal implicates the interaction between c-JUN and FOSL1 as necessary for survival of a subset for cancer cells. Each figure represents CRISPR screen of 342 cancer cell lines. Red boxes highlight cell lines with dependency scores ≤ -0.5 for that interaction. (A) FOSL1 gene effect (x-axis) and cJUN gene effect (y-axis). cJun/Fra1 dual-dependent cells are enriched in HNSCC, NSCLC, pancreatic cancer, melanoma, ovarian cancer, and glioma. (B) Combination of cFOS gene effect (x-axis) and cJUN gene effect (y-axis). No cancer cell lines exhibit dual dependency on cFOS and cJUN.

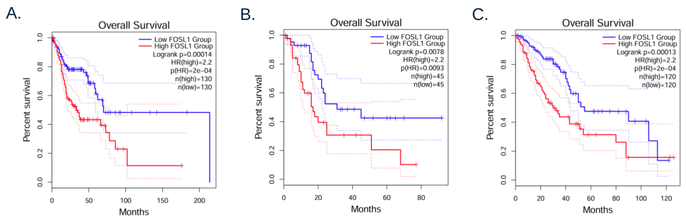


Figure 2. High FOSL1 expression is predictive of poor prognosis in several clinical cancer indications. Kaplan-Meier plots of overall survival were generated using quartile group cutoff (25% low; 75% high), 95% confidence interval (dotted line), high expression (red), low expression (blue) for the following cancer subtypes from the TCGA (KM curves generated using Gepia2). (A) Head and neck squamous cell carcinoma (HNSCC); (B) Pancreatic adenocarcinoma; and (C) Lung adenocarcinoma.

FraAP Mechanism of Action

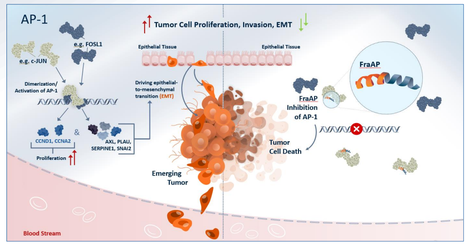


Figure 3. The AP-1 complex is a master regulator of proliferation/survival and EMT. Jun and Fra1 (FOSL1 gene product) heterodimerize to form the AP-1 complex in HNSCC and other cancers. AP-1 overactivation drives tumor cell proliferation and survival by regulating transcription of CCND1, CCNA2 and promotes epithelial-to-mesenchymal transition (EMT) via transcriptional regulation of AXL, PLAU, SERPINE1, and SNAI2. EMT is a process whereby tumor cells become more invasive, metastatic, and resistant to chemotherapy. FraAP is a peptide designed to disrupt AP-1 dimerization, thereby preventing AP-1 mediated transcription. The result is antagonism of oncogenic gene transactivation leading to selective tumor cell death and reduced tumor cell invasion.

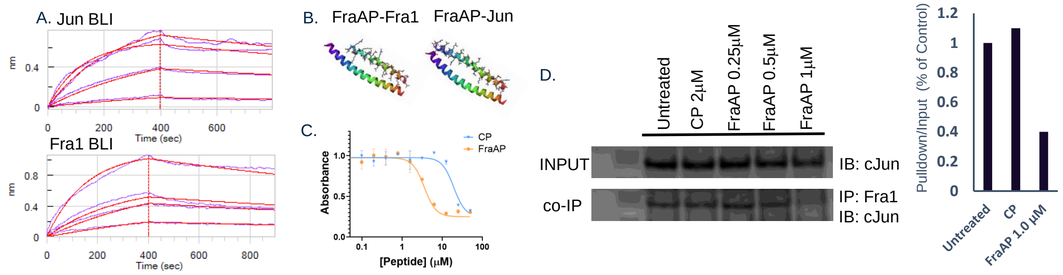


Figure 4. AP-1 antagonist peptide demonstrates target binding and disruption of Jun-Fra1 protein-protein interaction (PPI). A: Direct binding of FraAP to Jun (Kd=27.1nM) and Fra1 (Kd=36.0nM) by biofilm interferometry technology (BLI). B: AlphaFold and AlphaFold_ multimer version 1.5 model of FraAP binding to Fra1 and Jun. The best model was converted into D-aa peptide using python tools. A hybrid scoring function combining the Vina score and physicochemical similarity score is applied for model ranking. C: FraAP disrupts the binding of AP-1 complex to its target DNA. Fra1 and Jun proteins, in the presence and absence of increasing FraAP or control peptide (CP) concentration, were added to wells containing immobilized consensus-binding site oligo (5´-TGA(C/G)TCA-3´) and signal detected by ELISA. D: Co-immunoprecipitation of cJun/Fra1 in SW579 cells (JUN/FOSL1 dependent) disrupted following treatment with FraAP concentrations for 6hrs. CP had no impact under same conditions. Top row - Input, 10ug lysate IB: anti-Jun Rabbit mAB. Bottom row – IP: anti-Fra-1 Rabbit mAB, IB: anti-Jun Rabbit mAB. Quantification shown in bar graph.

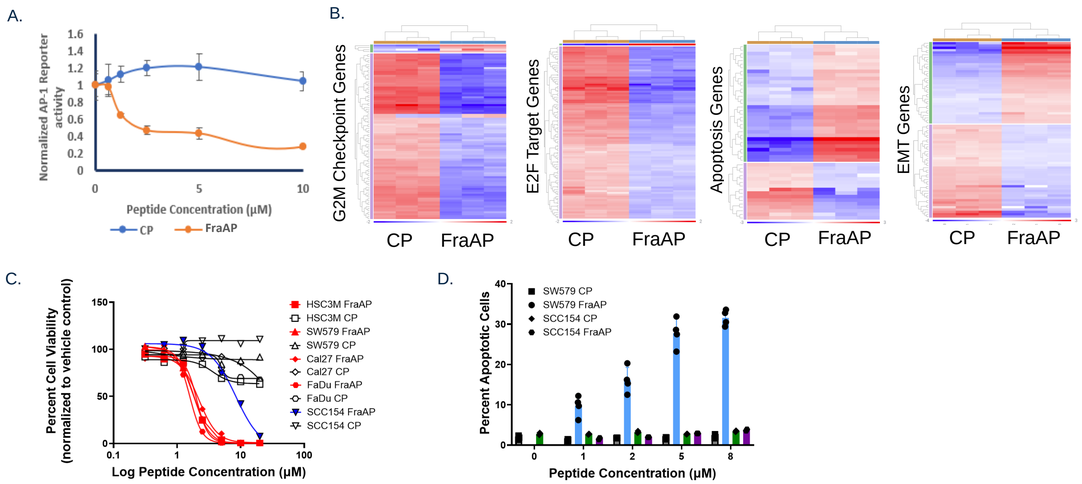
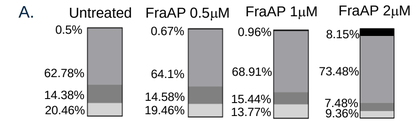


Figure 5. AP-1 antagonist peptide inhibits oncogenic pathways and induces apoptosis. A) FraAP inhibits AP-1 transcriptional activity in HEK293 AP-1 firefly luciferase reporter assay (IC50 of 1.13μM). Cells were treated with FraAP or CP for 24h. JNK was stimulated with 2.5nM PMA 2h after peptide. B) Hallmark pathway analysis of RNA sequencing data in SW579 cells indicates that FraAP promotes differential expression of genes involved in cell cycle/proliferation (G2M checkpoint, E2F target), apoptosis and EMT pathways. Each row of the heatmap indicates a differentially expressed gene (DEG) and each column represents treatment condition (n=2; each in triplicate). DEGs are significant in Q <0.05 and a linear fold-change ± 1.5. The heatmaps are color-coded on the basis of z-score. C) FraAP demonstrates potent cytotoxicity in AP-1 dependent HNSCC cells (HSC3M IC50=2.028μM; SW579 IC50=1.815μM; Cal27 IC50=2.192μM; FaDu IC50=1.583μM). AP-1 independent HNSCC cells display reduced sensitivity (SCC154 IC50=8.392μM). Cells were treated with increasing peptide concentration and viability was measured by CellTiter Glo 48-hrs post peptide treatment. D) Dose-dependent increase in percentage of apoptotic cells in SW579 but no impact in SCC154 (AP1 independent cell line). Cells were treated with increasing peptide concentration and annexin PI staining was detected by flow cytometry at 24-hrs post peptide treatment.



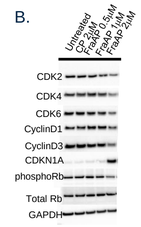
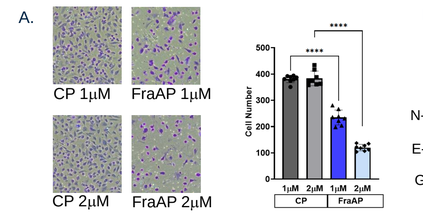


Figure 6. FraAP antagonizes proliferation of AP-1 dependent tumor cells. A, Cell-cycle analysis of SW579 cells exposed to FraAP or CP for 24 hours. Histograms represents PI staining as assayed by flow cytometry and indicate the percentage of cells in G1, S, or G2–M phase. B: Corresponding effect on cell cycle regulatory genes by treatment of SW579 cells with FraAP or CP for 24 hours as determined by immunoblot.



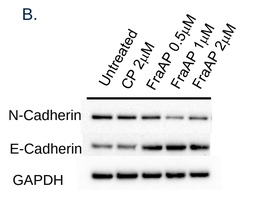


Figure 7. FraAP inhibits cancer cell invasion. A, SW579 cells treated with FraAP exhibit reduced invasion in an in vitro Matrigel coated Boyden chamber assay. **** indicates p <0.0001. B, FraAP treatment of SW579 induces a reduction in the mesenchymal marker N-Cadherin and an increase in the epithelial marker E-Cadherin by immunoblot.

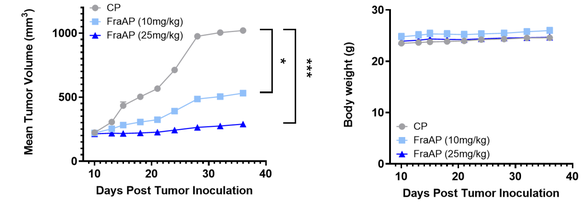


Figure 8. Anti-tumor activity of FraAP in HNSCC xenograft model. (Left) Tumor volumes of SW579 subcutaneous xenografts treated with control peptide (CP) or the indicated dose of FraAP administered 3x/week SC. (Right) FraAP is well tolerated and does not impact body weights over the course of the study. Error bars represent SEM. (n=5/group * indicates p<0.05, *** p<0.001).

Conclusions

The interaction of cJun and Fra1 to form the AP-1 complex is implicated in tumor cell proliferation, survival and metastasis. Antagonism of cJun and Fra1 dimerization and prevention of AP-1 complex formation with FraAP represents a novel approach to target AP-1 driven tumors.

- FraAP demonstrates target engagement with AP-1 family members, antagonizes cJun and Fra1 PPI and inhibits AP-1 transcriptional activity in vitro.
- FraAP inhibits AP-1 dependent cell survival, proliferation and invasion, and demonstrates potent in vitro cytotoxicity in cJun and Fra1-dependent HNSCC cells. Similar data has been generated in LUSC cells.
- FraAP demonstrates anti-tumor activity in vivo in an AP-1 dependent subcutaneous xenograft model.

These data demonstrate that FraAP disrupts AP-1 dimerization, preventing AP-1 mediated transcription and supports continued development of FraAP as a potential therapeutic option for AP-1 driven tumors.

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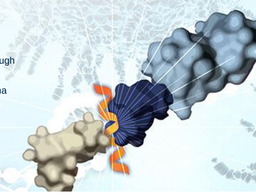

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