JunAP, a Peptide Antagonist of the Activator Protein 1 Transcription Factor, Demonstrates Cancer Cell Cytotoxicity and Reduced Invasion In Vitro and Tumor Regression In Vivo

K. Mendelson, Z. F. Mattes, S. Leong, R. Ramirez, M. Koester, C. Scuoppo, J. Diehl, E. Gallagher, J. Gonzales, F. Abbate, L. Ghamsari, G. Merutka, B. J. Kappel, A. Vainstein-Haras, J. A. Rotolo. Sapience Therapeutics, Tarrytown NY

SAPIENCE THERAPEUTICS

Poster #3051

Abstract

The activator protein 1 (AP-1) transcription factor is a dimeric complex consisting of proteins from the JUN, FOS, MAF, and ATF families. JUN proteins preferentially regulate genes that are implicated in proliferation and apoptosis, whereas FOS proteins are required for tumor angiogenesis and tumor invasion (Eferl, R. et al 2003). AP-1 complexes have been identified as novel therapeutic targets in cancer due to their role in tumor growth, invasion, metastasis, angiogenesis and chemoresistance. Dimerization of components of the AP-1 complex via leucine zipper domain interactions is required for DNA binding and subsequent transcriptional activity. We designed a peptide (Jun antagonizing peptide, JunAP) targeting the basic leucine zipper motif of AP-1 to antagonize AP-1 complex formation and prevent associated activity. Fluorescence polarization and DNA ELISA assays demonstrate target binding and selectivity of JunAP towards AP-1 family members, co-immunoprecipitation experiments reveal that JunAP blocks protein-protein interactions between cJun and its key binding partners, and reporter assays confirm inhibition of AP-1 transcriptional activity in vitro. Pathway analysis of RNA sequencing data confirmed by qPCR analysis shows that JunAP disrupts key pathways required for cell survival and migration. Corresponding functional in vitro assays reveal that JunAP exhibits an inhibitory effect on invasion in Boyden chamber assays and demonstrates potent cytotoxicity in Jun-dependent cells. Further, in two independent subcutaneous xenograft models, BT549 triple negative breast cancer and WM3211 melanoma, administration of JunAP results in tumor regression, demonstrating the anti-cancer potential of targeting the AP-1 complex. In summary, these data support JunAP 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 interaction between cJUN and FOSL1 (gene name for Fra1 protein) as crucial in cancer cell survival. We focused on the role of disrupting the interaction between cJUN and FOSL1 with a peptide (JunAP) targeting the basic leucine zipper motif of AP-1 to antagonize AP-1 complex formation.

A. cJUN/FOSL1 **B.** cJUN/cFOS **C.** cJUN/FOSL2 **D.** cJUN-FOSB

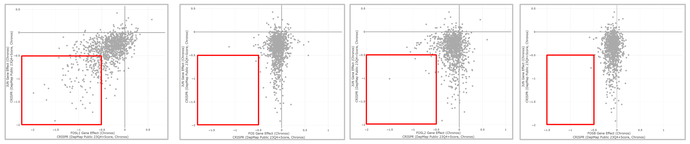


Figure 1: DepMap portal implicates the interaction between cJUN and FOSL1 as crucial in cancer cell survival. Each figure represents Crispr screen of 342 cancer cell lines. Red boxes highlight all cell lines with dependency score of <= -0.5 **(A)** FOSL1 gene effect (x-axis) and cJun gene effect (y-axis). (**B**) cFOS gene effect (x-axis) and cJun gene effect (y-axis). **(C)** FOSL2 gene effect (x-axis) and cJun gene effect (y-axis). (**D**) FOSB gene effect (x-axis) and cJun gene effect (y-axis).

A. **B.** **C.**

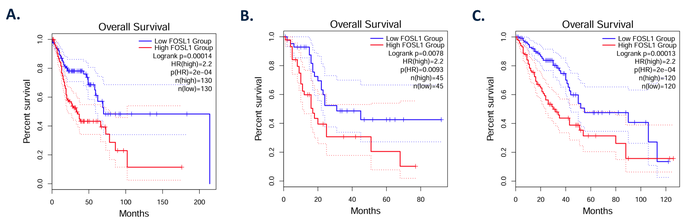


Figure 2: High FOSL1 expression is predictive of lower overall survival in numerous clinical cancer indications. Kaplan-Meier plots of overall survival were generated using quartile group cutoff (25% low; 75% high), 95% confidence interval (dotted line), high expression (red), low expression (blue) for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

JunAP Mechanism of Action

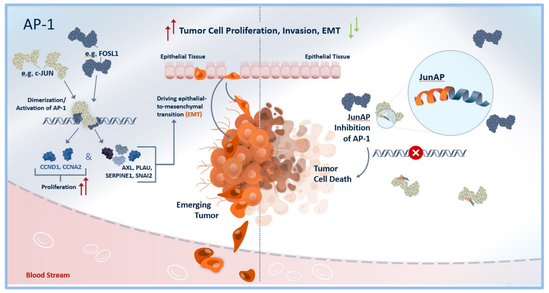


Figure 3: The AP-1 complex is a master regulator of proliferation, survival and EMT. cJun and Fos-related antigen 1 (FOSL1 gene; Fra1 protein) heterodimerize to form the AP-1 complex. AP-1 overactivation in cancer drives tumor cell proliferation and survival by regulating transcription of CCND1, CCNA2 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. JunAP 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.

A. **B.** **C.**

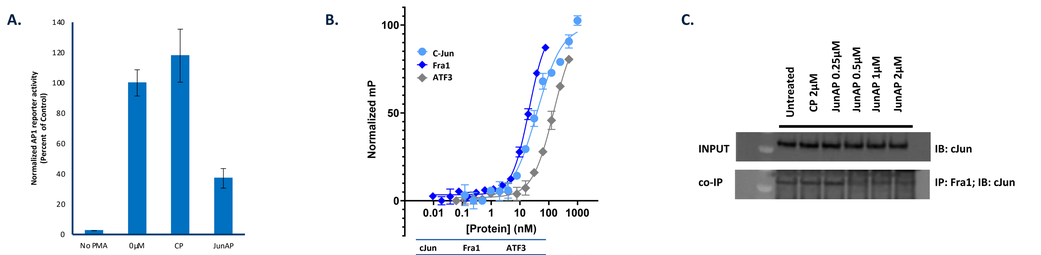


Figure 4: AP-1 antagonist peptide demonstrates inhibition of AP-1 transcriptional activity, target binding, and selectivity *in vitro*. A) Reporter assay in HEK293 cells stably expressing luciferase from TRE sequences and stimulated with PMA indicates antagonism in cells treated with 5 µM JunAP but not control peptide (CP). **B)** Fluorescence polarization assay indicates peptide binding/association preference for cJun and Fra1 over leucine zipper protein ATF3. **C)** Co-immunoprecipitation reveals that JunAP blocks protein-protein interactions between cJun and FOSL1. Top - Input, 10ug lysate IB: anti-Jun Rabbit mAB. Bottom – IP: anti-Fra-1 Rabbit mAB, IB: anti-Jun Rabbit mAB.

A. **B.** **C.** **D.**

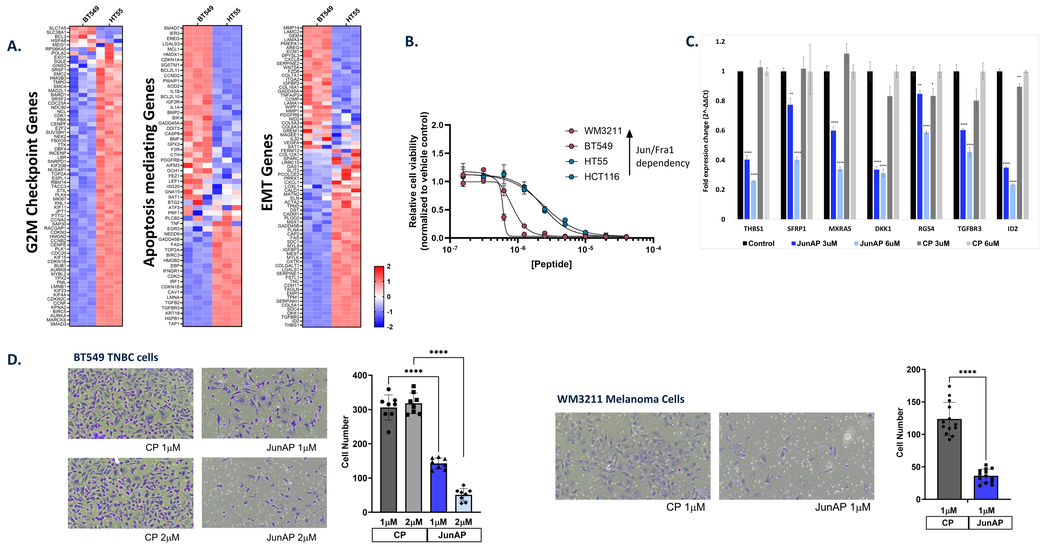


Figure 5: AP-1 antagonist peptide inhibits oncogenic and EMT pathways. A) GSEA Hallmark pathway analysis of RNA sequencing data in TNBC BT549 cell line (JUN/FOSL1 dependent) and HT55 colorectal adenocarcinoma (JUN/FOSL1 independent) indicates that JunAP promotes differential expression of genes involved in proliferation (G2M checkpoint), apoptosis and EMT pathways. Each row of the heatmap indicates a differentially expressed gene and each column represents a cell line (n=2; each in triplicate). Differentially expressed genes are significant in Q <0.05 and a linear fold-change ± 1.5. The heatmaps are color-coded on the basis of z-scores. **B)** JunAP demonstrates potent cytotoxicity in AP-1 dependent cells in a 48 hour viability assay. Cells are ordered from greatest JUN/FOSL1 dependency based on DepMap portal. **C)** Top EMT genes downregulated following JunAP treatment in BT549 TNBC cells by RNASeq were validated by qPCR. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p <0.0001 **D)** JunAP demonstrates inhibitory effect on invasion. BT549 TNBC cells and WM3211 melanoma cells treated with JunAP exhibit reduced invasion in an *in vitro* Matrigel coated Boyden chamber assay. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p <0.0001

A. BT549 TNBC cells **B.** WM3211 Melanoma Cells

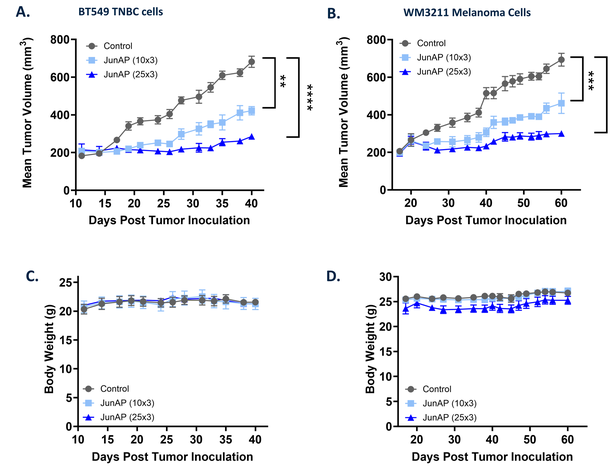


Figure 6. AP-1 antagonist peptide promotes anti-tumor response in two independent tumor models. Mean tumor volumes for the indicated treatment cohorts **(A)** BT549 TNBC and **(B)** WM3211 melanoma indicate enhanced anti-tumor activity in animals receiving JunAP in a subcutaneous xenograft model. JunAP resulted in 45.9% and 78.3% tumor growth inhibition (TGI) in BT549 tumors at 10 and 25 mg/kg dose levels, respectively. JunAP resulted in 57.29% and 85.7% TGI in WM3211 tumors at 10 and 25 mg/kg dose levels, respectively. Error bars represent SEM. n=5/group. ** = p<0.01, *** = p<0.001, **** = p <0.0001. JunAP treatment is well tolerated in vivo and does not induce loss of body mass in **(C)** BT549 and **(D)** WM3211 subcutaneous xenograft models.

Conclusions

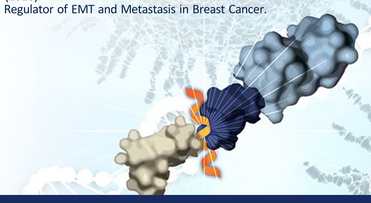
The interaction of cJun and Fra1 to form the AP-1 complex is implicated in the survival of diverse cancer cells.

Disruption of cJun and Fra1 dimerization and subsequent prevention of AP-1 complex formation represents a novel approach to target AP-1 driven tumors.

- **JunAP demonstrates selective target engagement with AP-1 family members, blocks cJun and Fra1 protein-protein interactions and inhibits AP-1 transcriptional activity in vitro.**
- **JunAP inhibits AP-1-dependent cell survival and invasion, and demonstrates potent in vitro cytotoxicity in cJun-dependent cells.**
- **JunAP demonstrates anti-tumor activity in vivo in BT549 triple negative breast cancer as well as WM3211 melanoma subcutaneous xenograft models.**

These data support a paradigm in which JunAP disrupts AP-1 dimerization, preventing AP-1 mediated transcription. The result is antagonism of oncogenic gene transactivation leading to selective tumor cell death and reduced tumor cell invasion.

References:
Eferl, R., Wagner, E. AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 3, 859–868 (2003).
Talotta, F., Casalino, L., Verde, P. The nuclear oncoprotein Fra-1: a transcription factor knocking on therapeutic applications' door. *Oncogene* 39:4491–4506 (2020).
Casalino, L., Talotta, F., Matino, I., Verde, P. FRA-1 as a Regulator of EMT and Metastasis in Breast Cancer. Int. J. Mol. Sci. 24(9), 8307 (2023).



Contact information: jrotolo@sapiencetherapeutics.com