β-catenin antagonist peptide attenuates oncogenic gene transactivation and promotes antitumor activity in breast cancer models

Jim A. Rotolo[1], Lila Ghamsari[1], Erin Gallagher[1], Ricardo Ramirez[1], Mark Koester[1], Siok Leong[1], Bram Herpers[2], Gene Merutka[1] and Barry J. Kappel[1]

[1]Sapience Therapeutics, 500 Mamaroneck Avenue Suite 320, Harrison NY 10528; [2]Ocell0 B.V., JH Oortweg, 21, 2333 CH Leiden, The Netherlands

Contact information: jrotolo@sapiencetherapeutics.com

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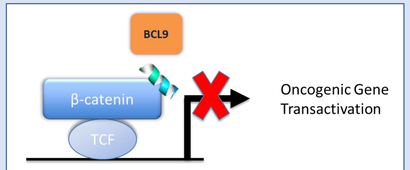
Introduction

Sapience Therapeutics is focused on discovering and developing peptide-based therapeutics to previously 'undruggable' targets for major unmet medical needs, particularly high mortality cancers. Our peptide candidates target protein-protein interactions (PPIs) responsible for oncogenic transcription or dampening the immune response to tumors.

Canonical Wnt signaling pathway has historically been considered an "undruggable" cancer target, as small molecule approaches have proven ineffective or toxic. Activating mutations in the Wnt/β-catenin signaling pathway drive cancer initiation and contribute to tumor growth, angiogenesis and metastasis in many solid tumors and hematological malignancies. BCL9 is a co-factor of β-catenin that promotes tumor progression by conferring enhanced proliferative, metastatic, and angiogenic properties to cancer cells.



β-catenin antagonist peptides occupy oncogenic co-factor binding sites, disrupting gene transactivation

Antagonism of the BCL9/β-Catenin interaction suppresses oncogenic Wnt gene transactivation, while not disrupting interactions with destruction complex components such as APC or factors involved in β-catenin homeostatic functions such as TCF4 and E-cadherin. Sapience has generated cell-penetrating β-catenin antagonist peptides designed to inhibit its interaction with BCL9 and impair tumor cell proliferation, metastasis, and angiogenesis

Abstract

Aberrant activation of the canonical Wnt signaling pathway occurs in a variety of cancers, resulting in overactivation of β-catenin-mediated transcriptional activity and increased expression of genes that promote tumor survival, proliferation and inhibit differentiation. Disruption of the β-catenin transcription complex, by inhibiting protein-protein interactions required for its formation, represents a powerful approach to inhibit this previously 'undruggable' target. Here, we characterize the anti-tumor activity of β-catenin antagonist peptides, BCA1 and BCA2, designed to disrupt the β-catenin transcription complex, in breast cancer models. In in vitro experiments, MCF7 (HER2^{neg}/HR^{pos}) breast adenocarcinoma cells were exposed to β-catenin antagonist peptides, and the impact on β-catenin-mediated gene transactivation was determined by quantitative polymerase chain reaction (qPCR). Results indicate that β-catenin antagonist peptide attenuated oncogenic gene transactivation in MCF7 cells, significantly decreasing the expression of direct β-catenin target genes including TCF7, FOX01 and CTNNB1 ($p<0.05$). The impact of β-catenin antagonist peptides on cancer cell viability was quantified by annexin V/PI flow cytometry analysis, indicating a dose-dependent decrease in overall cell viability 48 hours post peptide exposure, with the most effective peptide demonstrating an EC_{50} value of 840 nM. Additionally, BCA1 was administered to primary breast cancer tumoroids (Her2^{pos}/HR^{neg} and Her2^{neg}/HR^{neg}), and the impact on tumoroid viability was quantified by histology and immunofluorescence microscopy. β-catenin antagonist peptide BCA1 resulted in dose-dependent decrease in surviving Her2^{pos}/HR^{neg} and Her2^{neg}/HR^{neg} breast cancer tumoroids, with EC_{50} values of 5.2 and 6.9 μM, respectively. Finally, the impact of BCA2, an analogue of BCA1 with an additional N-terminal modification, on subcutaneous MCF7 xenograft tumors was determined in vivo. Administration of 5 mg/kg BCA2 via subcutaneous injection at a dosing frequency of 3x/week for 3 weeks resulted in 18-day tumor regression from the start of treatment and a 63.1% tumor growth inhibition compared to vehicle-treated controls ($p<0.05$). Taken together, these data demonstrate the significant anti-breast cancer activity of β-catenin antagonist peptides in vitro and in vivo.

Results

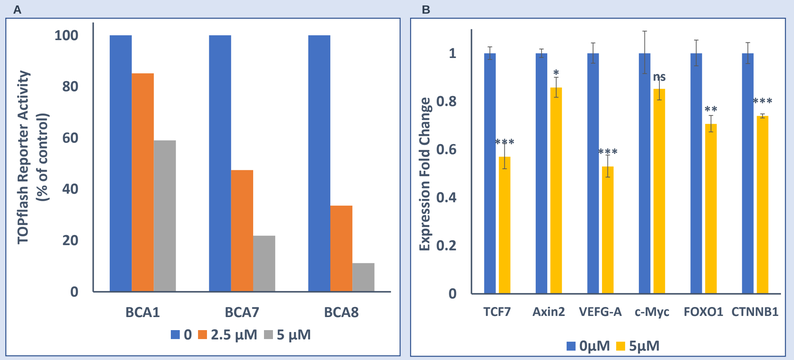


Figure 1: β-catenin antagonist peptides inhibit Wnt transcription. A) β-catenin antagonist peptides BCA1, BCA7 and BCA8 were analyzed by TOPflash Wnt pathway-responsive reporter assay. BCA1 represents an early peptide design, and resulted in an IC_{50} value of 5.3 μM in this assay. Modified peptides BCA72 and BCA77 show dose-dependent inhibition of reporter activity, with IC_{50} values of 2.3 and 1.8 μM, respectively, in this assay. B) BCA1 antagonizes Wnt transcription in vitro. MCF7 Her2^{neg}/HR^{pos} breast cancer were administered peptide for 6 hrs, and gene expression was determined by qPCR. Gene expression is normalized to β-actin and represented as percent of vehicle control

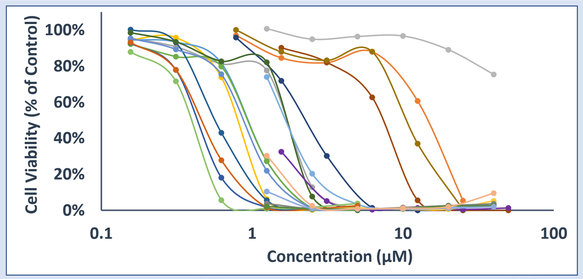


Figure 2: Structure activity relationship (SAR) analysis of β-catenin antagonist peptide BCA1. A series of peptides were designed to incorporate amino acid substitutions to the base BCA1 sequence to improve target affinity, manufacturability, stability and reduce potential immunogenicity. A total of 85 peptides were synthesized, with 84 soluble in aqueous buffer and tested for biologic activity. Data represents cytolytic activity of the panel of peptides against HL60 promyelocytic leukemia cells. Cells were administered peptide for 48 hrs prior to flow cytometric analysis following annexin V/propidium iodide staining. SAR analysis resulted in EC_{50} range of peptide candidates from 288 nM to >40 μM.

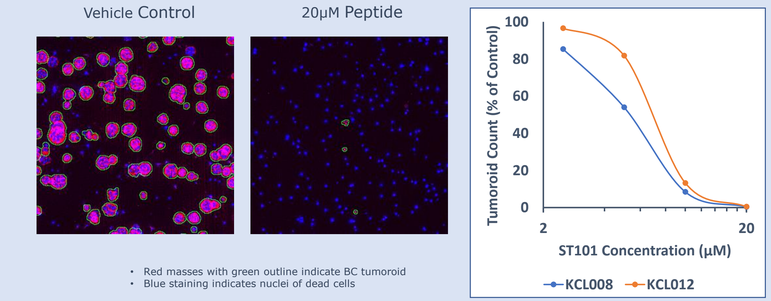


Figure 3: BCA1 displays significant anti-tumor activity in PDX tumoroids in vitro. PDX tumoroids were grown from KCL008 (Her2^{pos}/HR^{neg}) or KCL012 (Her2^{neg}/HR^{neg}) breast cancer cells in 3-dimensional culture. 24 hrs post culture, cells were administered BCA1 peptide (0-20 μM), and 7 days later tumoroids were quantified by scanning microscopy following nuclei and actin cytoskeleton staining with Hoechst and Rhodamine-Phalloidin, respectively. A) Representative images from vehicle and BCA1 peptide treated tumoroids. Pink masses indicate surviving tumoroids; Blue staining indicates nuclei of dead cells. B) Quantification of surviving tumoroids after 7 days of culture in the presence of BCA1. BCA1 resulted in EC_{50} of 5.2 and 6.9 μM against KCL008 and KCL012 tumoroids, respectively. The decrease in tumoroid count was attributed to peptide cytotoxic activity.

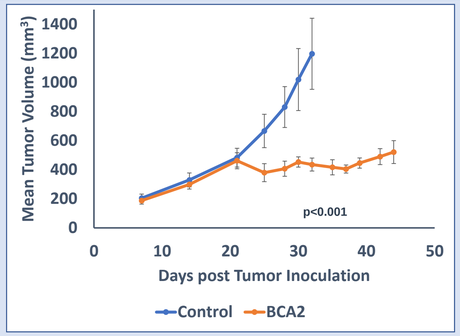


Figure 4: BCA2 displays significant anti-tumor activity in MCF7 (Her2^{neg}/HR^{pos}) breast cancer xenograft model. Nude mice with implanted 60 day estradiol slow release pellet were inoculated with $2x10^6$ MCF7 cells in Matrigel. Mice were administered 5 mg/kg BCA2 peptide 3x/week over three weeks by subcutaneous injection starting on day 21 post tumor inoculation. Tumor volume was monitored 3x/week. Peptide exposure resulted in tumor regression and a significant tumor growth delay compared to vehicle ($p<0.001$). Data points represent mean ±SE for n=8/group.

Conclusions

- β-catenin antagonist peptides inhibit Wnt transcription, as determined by TOPflash reporter assay and qPCR analysis of MCF7 breast cancer cells exposed to BCA1 peptide.
- SAR analysis was performed to improve β-catenin antagonist peptide biologic activity and manufacturability. Peptide EC_{50} was improved to 288 nM.
- Early candidate BCA1 demonstrates cytotoxic activity against patient-derived Her2^{pos}/HR^{neg} or Her2^{neg}/HR^{neg} breast cancer tumoroids. EC_{50} values of 5-7 μM were observed for BCA1 in this assay.
- Tumor regression and significant tumor growth delay (TGD) was observed in MCF7 breast cancer subcutaneous xenograft tumor model. Dosing was initiated on day 21 with tumors ~480 mm^3.
- Potent activity of BCA7 and BCA8 in the Topflash reporter assay suggest that these peptides will demonstrate enhanced activity in in vitro and in vivo cytotoxicity assays.
- These peptides represent a novel therapeutic approach for many difficult to treat cancers, including CRC, breast cancer, pancreatic cancer, HNSCC, MM, and MLL.