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

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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.

Cancerous Wnt signaling pathway has historically been considered an ‘undruggable’ cancer target, as small molecule approaches have proven ineffective or toxic. Activations in the Wnt/b-catenin pathway drive cancer initiation and contribute to tumor growth, angiogenesis and metastasis in many solid tumors and hematological malignancies. As a consequence, the 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 transcription

Aβ-catenin 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 vitro experiments, MCF7 (HER2+/ER+), breast adenocarcinoma cells were exposed to β-catenin antagonist peptides, and the impact on β-catenin-mediated gene transactivation was quantitated by quantitative polymerase chain reaction (qPCR).

Antagonism of the BCL11B-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 homoeostatic functions such as TCF4 and E-cadherin. Sapience has generated cell- penetrating β-catenin antagonist peptides designed to inhibit its interaction with BC1L and impair tumor cell proliferation, metastasis, and angiogenesis.

β-catenin antagonist peptides inhibit Wnt transcription

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 vitro experiments, MCF7 (HER2+/ER+), breast adenocarcinoma cells were exposed to β-catenin antagonist peptides, and the impact on β-catenin-mediated gene transactivation was quantitated by quantitative polymerase chain reaction (qPCR).

Results

β-catenin antagonist peptides inhibit Wnt transcription. A) β-catenin antagonist peptides BCA1, BCA7 and BCA8 were analyzed by TopFlash Wnt pathway reporter assay. BCA1 represents an early peptide design, and results in an E<sub>C0</sub> value of 5.3 µM for this assay. Modified peptide BCA7 and BCA8 show dose-dependent inhibition of reporter activity, with E<sub>C0</sub> values of 2.3 and 1.8 µM, respectively. In this assay, BCA9 antagonizes Wnt transcription in vitro. MCF7 her2+/ER+ 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.

Discussion Table of values (mean ± SEM) represents results for BCA1, BCA2, BCA7, and BCA8. Values are expressed as a percentage of control. Significant differences were assessed by Student’s t-test. 

β-catenin antagonist peptides were evaluated by measuring changes in β-catenin and MMP-2 protein levels relative to controls and normalized to β-actin. In this assay, BCA1, BCA2, and BCA8 exhibit significant decreases in β-catenin expression compared to controls. However, BCA7 and BCA8 demonstrate no significant effect on β-catenin expression. BCA8 results in a significant decrease in MMP-2 expression compared to controls. No significant changes were observed for BCA1 and BCA2.

Figure 1: β-catenin antagonist peptides inhibit Wnt transcription. A) β-catenin antagonist peptides BCA1, BCA7 and BCA8 were analyzed by TopFlash Wnt pathway reporter assay. BCA1 represents an early peptide design, and results in an E<sub>C0</sub> value of 5.3 µM for this assay. Modified peptide BCA7 and BCA8 show dose-dependent inhibition of reporter activity, with E<sub>C0</sub> values of 2.3 and 1.8 µM, respectively. In this assay, BCA9 antagonizes Wnt transcription in vitro. MCF7 her2+/ER+ 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.

Table 1: β-catenin antagonist peptides inhibit Wnt transcription. A) β-catenin antagonist peptides BCA1, BCA7 and BCA8 were analyzed by TopFlash Wnt pathway reporter assay. BCA1 represents an early peptide design, and results in an E<sub>C0</sub> value of 5.3 µM for this assay. Modified peptide BCA7 and BCA8 show dose-dependent inhibition of reporter activity, with E<sub>C0</sub> values of 2.3 and 1.8 µM, respectively. In this assay, BCA9 antagonizes Wnt transcription in vitro. MCF7 her2+/ER+ 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.

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 identify β-catenin antagonist peptide biologic activity and manufacturability. Peptide E<sub>C0</sub> was improved to 288 nM.
• Early candidate BCA1 demonstrates cytotoxic activity against patient-derived Her2+/ER+ and Her2+/ER+ breast cancer tumor buds. E<sub>C0</sub> values of 5.7 µM were observed for BCA1 in this assay.

Figure 2: Structure activity relationship (SAR) analysis of β-catenin antagonist peptide BCA1. A series of peptides were designed to incorporate optimization and substitutions in the beta-tumor necrosis factor receptor surface stability, manufacturability, and reduce potential immunogenicity. A total of 88 peptides were synthesized, with 94 cycles in sequence length and tested for biologic activity. Data represents cytotoxic activity of the panel of peptides against HUH7, 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 E<sub>C0</sub> values of peptide candidates from 288 nM to 10 µM.

Figure 3: BCA1 displays significant anti-tumor activity in PDX tumors in vivo. PDX tumors were grown from UCC1026 (Her2+/ER+) or UCC1202 (Her2+/ER+) breast cancer cells in 3-dimensional culture. 24 hrs post culture, cells were administered BCA1 peptide (5-20 µM), and 7 days later tumors were quantified by scanning microscopy following nucleic and antigen cytopathology staining with trichoch and Rhodamine Phalloidin, respectively. A) Representative images of vehicle and BCA1 peptide treated tumors. Pink masses indicate surviving tumors. Blue staining indicates nucleoli of dead cells. B) Quantification of surviving tumors after 7 days of culture in the presence of BCA1. BCA1 results in E<sub>C0</sub> of 5.2 and 6.9 µM against UCC1026 and UCC1202 tumors, respectively. The decrease in tumor count was attributed to peptide cytotoxic activity.

Figure 4: BCA2 displays significant anti-tumor activity in MCF7 (Her2+/ER+) breast cancer xenograft model. Nude mice with implanted 60 day established slow growth pellets were inoculated with 2×10<sup>6</sup> MCF7 cells in Matrigel. Mice were administered BCA2 peptide twice a week, and tumors were quantified by ear perimeter ultrasonography on day 20 after inoculation. Tumor volume was measured 3x/week. Peptide exposure resulted in tumor regression and a significant tumor growth delay compared to vehicle [p<0.001]. Data points represent mean ± SEM for n/group.

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