Cell penetrating peptide, ST101, disrupts ATF5 regulation of anti-apoptotic Bcl-2 family proteins, resulting in induction of cancer cell death in vitro and tumor growth inhibition/regression in vivo

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ABSTRACT

Anti-apoptotic B cell lymphoma 2 (Bcl-2) family proteins are frequently overexpressed across a variety of tumors, resulting in tumor cell survival and resistance to therapy. Inhibition of the expression or activity of these survival factors is an attractive approach for cancer therapy. Activating transcription factor 5 (ATF5) regulates gene transcription of anti-apoptotic Bcl-2 family proteins in neural progenitor cells and a wide range of human cancer cells. Here, we describe ST101, a rationally designed, synthetic, D-amino acid, cell penetrating peptide therapeutic designed to disrupt the protein-protein interactions driving ATF5-regulated gene transcription. Exposure of HL60 promyelocytic leukemia cells and MCF7 breast adenocarcinoma cells to low micromolar concentration of ST101 resulted in a decrease in MCL-1, BCL-2 and BIRC5 (Survivin) mRNA expression at 4 and 24 hrs post exposure. Further, exposure to ST101 resulted in a dose-dependent loss of viability across a panel of human cancer cells, including MCF7, HL60, U251 glioblastoma, A375 melanoma, DU145 prostate cancer, and A549 lung adenocarcinoma, characterized by an increase in annexin V and PI staining by flow cytometry peaking 48 hrs post exposure, resulting in a median half maximal effective concentration (EC50) value of 2.9 µM. In contrast, normal human peripheral blood mononuclear cells and bone marrow mononuclear cells were resistant to ST101-induced cell death, with >80 µM EC50 values. In mouse xenograft experiments, 25mg/kg ST101 administered three times per week for three weeks resulted in significant tumor regression in MCF7 and U251 subcutaneous tumors as well as tumor growth delay in HL60 subcutaneous tumors. Tumor growth remained significantly inhibited weeks after the last treatment in the MCF7 and U251 models. In summary, ST101 selectively kills cancer cells, in part by decreasing BCL-2 family gene expression, resulting in significant reductions in tumor growth in mouse models. Taken together, these data validate ST101 as a potent peptide therapeutic candidate for a variety of solid tumor and hematologic malignancies.

ST101 Mechanism of Action

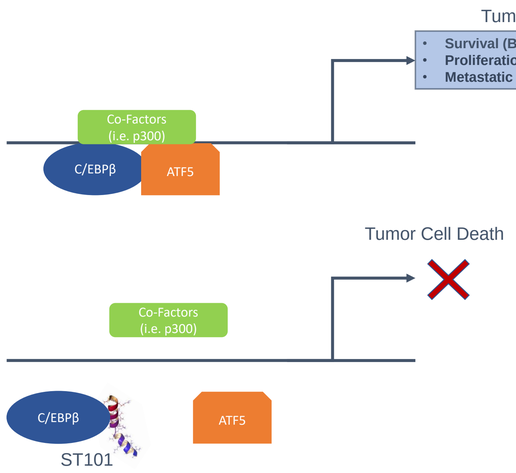


Figure 1: ST101 inhibits oncogenic transcription complex interactions. A) Graphic depicts interactions of oncogenic transcription factor ATF5 with C/EBPβ to promote expression of factors involved in cancer cell survival (Bcl-2 family of proteins), proliferation (cyclin D, Egr1) and metastatic potential (Integrin β1). B) ST101 associates with C/EBPβ via leucine zipper domain interactions, antagonizing the interaction of C/EBPβ with ATF5, and preventing heterodimer formation and associations with DNA regulatory elements. The result of ST101 exposure is inactivation of transcription of oncogenic genes (BCL-2, MCL-1, etc.) and selective tumor cell death.

ST101 In Vitro Activity

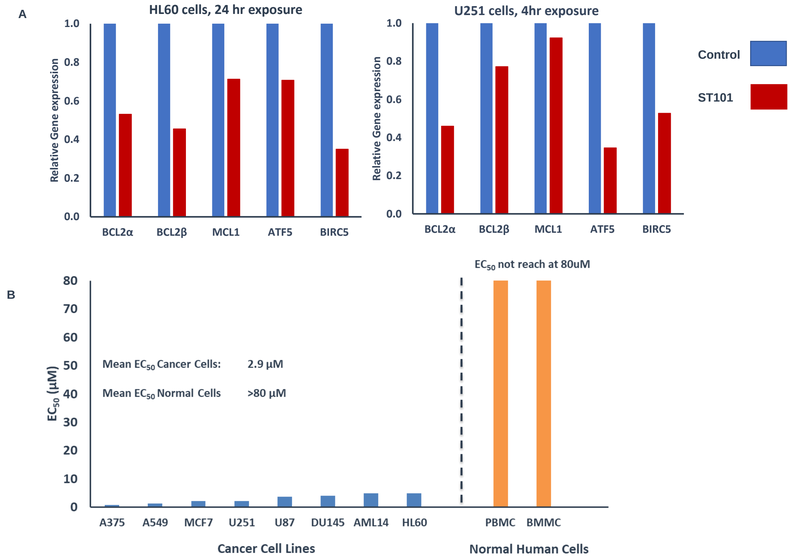


Figure 2: In vitro characterization of ST101 anti-tumor activity. A) RT-PCR performed on RNA from cells exposed to 20 µM ST101 for 4 or 24 hrs. Gene expression normalized to β-actin and represented as percent of vehicle control. B) Cytotoxicity assessed by AnnexinV/PI flow cytometry assay 48 hrs following ST101 exposure. Mean EC_{50} for cancer cell cytotoxicity is 2.9 µM (blue bars). Normal human PBMCs and BMMCs (orange bars) display reduced sensitivity to ST101-induced cytotoxicity and fail to reach an EC_{50} value in this dose range .

ST101 In Vivo Activity

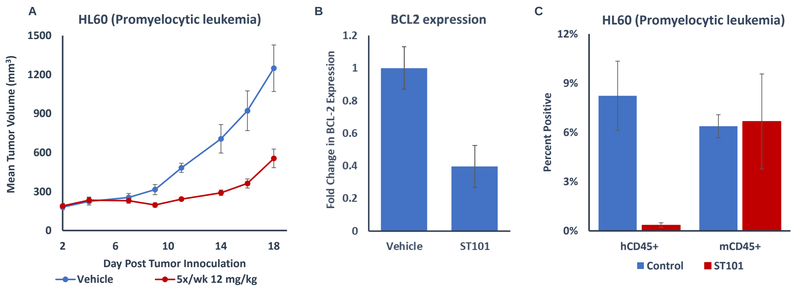
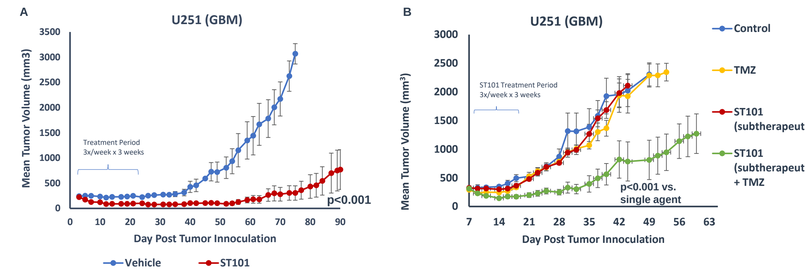


Figure 3: ST101 displays significant anti-tumor activity in HL60 cell models. A) Nu/J mice inoculated with $2x10^6$ HL60 cells in Matrigel were administered ST101 by subcutaneous injection. Tumor volume was monitored 3x/week. B) RNA was extracted from tumor-bearing mice exposed to 50 mg/kg ST101 on days 11-13, and analyzed for BCL-2 gene expression by RT-QPCR analysis. Data represents mean±SE of 3 tumor samples. C) Mice inoculated with $5x10^6$ HL60 cells by tail-vein infusion were administered 25 mg/kg ST101 3x/week. On day 21 post inoculation, bone marrow was collected and processed to determine percent of live, mononuclear cells stained positive for human CD45 by flow cytometry. Data represents mean±SE of 3 mice per group.

ST101 In Vivo Activity Cont'd



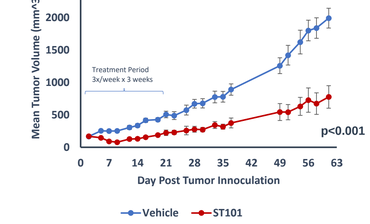


Figure 4: ST101 displays significant anti-tumor activity in U251 and MCF7 xenograft models. A) NOD/SCID mice inoculated with $2x10^6$ U251 cells in Matrigel were administered 50 mg/kg ST101 by a regimen of 3x/week over three weeks by subcutaneous injection starting on day 2 post tumor inoculation. Tumor volume was monitored 3x/week. ST101 exposure resulted in significant tumor growth delay compared to vehicle (p<0.001). B) Mice were inoculated as in A, and exposed to vehicle (blue line), subtherapeutic ST101 (10 mg/kg ST101 by oral gavage 3x/week over three weeks; red line), temozolomide (100 mg/kg 3x over one week; yellow line) or ST101 + temozolomide combination (green line); all treatments initiated on day 7 post tumor inoculation. Tumor volume was monitored 3x/week. Combination of subtherapeutic ST101 with temozolomide resulted in significant tumor growth delay compared to either single agent alone (p<0.001). C) Nude mice with implanted 60 day estradiol slow release pellet were inoculated with $2x10^6$ MCF7 cells in Matrigel. Mice were administered 25 mg/kg ST101 by a regimen of 3x/week over three weeks by oral gavage starting on day 2 post tumor inoculation. Tumor volume was monitored 3x/week. ST101 exposure resulted in significant tumor growth delay compared to vehicle (p<0.001). Data points represent mean ±SE for n=8/group.

CONCLUSIONS

- ST101 is a novel therapeutic agent with potential to treat many oncology indications.
- Cancer cells exposed to ST101 have decreased gene expression of pro-survival factors Bcl-2, Mcl-1 and Birc5 as demonstrated by RT-PCR and QPCR analyses.
- ST101 demonstrates tumor specific cell kill across a variety of tumors; Mean EC_{50} is 2.9 µM across a panel of melanoma, lung adenocarcinoma, breast adenocarcinoma, glioblastoma, prostate cancer and leukemia cell lines.
- Significant tumor growth delays (TGD) observed in multiple tumor models:
  - HL60 model p<0.05; 5-7 day TGD vs. vehicle control
  - U251 model p<0.001; Approx. 65 day TGD vs. vehicle control; 50% tumor cure
  - MCF7 model p <0.001; Sustained tumor regression
- Combination with temozolomide (TMZ) provides significant TGD in U251 xenograft model with p<0.001 vs. either single agent alone; approx. 17 day TGD vs. either monotherapy