SOR-C13, a novel cancer peptide therapeutic targeting the TRPV6 oncochannel, shows efficacy in breast and ovarian cancer xenografts in a murine model.

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ABSTRACT: Validation of new therapeutic targets in oncology has taken on a new urgency in an era of tumour-targeted drugs. TRPV6 has been suggested as a druggable target for 15 years but suitable inhibitors of this calcium channel have not been available. A peptide therapeutic drug antagonistic to TRPV6 is currently under development in a Phase I clinical trial. Pre-clinical data presented here show that targeting TRPV6 with SOR-C13 results in reduced growth in breast (T-47D) and ovarian (SKOV-3) xenografts in mice. These cell lines and the xenografts produced from them over-express TRPV6. Treatment (i.p.) with the peptide SOR-C13 derived from soricidin inhibits tumour growth compared to controls and with similar efficacy as positive controls of paclitaxel (breast) and carboplatin plus paclitaxel (CAT; ovarian). Combinations of peptide plus paclitaxel (for breast) and peptide plus CAT (for ovarian) showed improved efficacy over standard drug alone. These results support the clinical development of SOR-C13 as an anti-cancer therapeutic. We conclude that TRPV6 is a viable oncology target in epithelial tumours since its inhibition results in tumour growth suppression in these two models.

Introduction

Many epithelial cancers over-express TRPV6, a non-voltage gated calcium channel (Zhuang et al., 2002) now classified as an oncochannel. Inhibition of TRPV6 channel activity is anti-neoplastic. One such inhibitor is a peptide (SOR-C13) a member of a library of C-peptides from the C-terminus of the parent paralytic peptide (soricidin) isolated from the saliva of the Northern Short-tailed shrew (*Blarina brevicauda*).

Early *in vitro* work cited a number of epithelial cancer cell lines (breast, ovarian, prostate, lung, kidney, etc.) that showed reduced viability after treatment with peptides antagonistic to TRPV6 (Bowen et al., 2013). Here we validate TRPV6 targeting in murine xenograft models of breast and ovarian cancers reduce tumour growth rates.

Materials & Methods

Cell culture: Ovarian (SKOV-3) and breast (T-47D) cancer cells were cultured following protocols from ATCC at 37˚C and 5% CO_2.

Western Blotting: Quantification of TRPV6 in cell lines and in xenografted using a rabbit anti-TRPV6 antibody (Santa Cruz, H-90) with a goat anti-rabbit IgG-HRP secondary antibody (Santa Cruz) was used to develop the blot after blocking. The band intensity (Integrated Density Value) was determined with the imager software with autocorrect.

Xenograft. Female NOD-SCID mice (6 – 8 week old, 20 – 25 g) were purchased from JAX® Mice, Clinical & Research Services (Jackson Laboratory, Bar Harbor, Maine 04609 USA). SKOV-3 cells (1.5 x 10^6 cells /graft) were injected into 4 subcutaneous sites on the backs of female mice to establish the xenografts. T-47D cells (1 x 10^7 /graft) were injected into both fat pads of female mice to establish the xenografts.

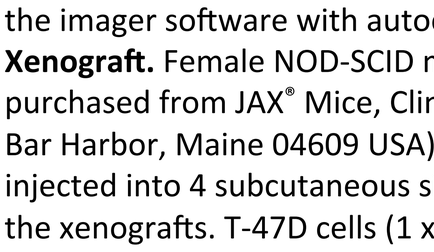
Peptide: SOR-C13 (KEFLHPSKVDLPR); 98% purity, 83% peptide content.

Dose response: Mice with SKOV-3 tumours were injected i.p. daily (400, 600 & 800 mg/kg) for 12 days. T-47D xenografts were treated (i.p. injection) daily, for 12 days with 200, 300 or 600 mg/kg followed by a 2 week no-treatment period.

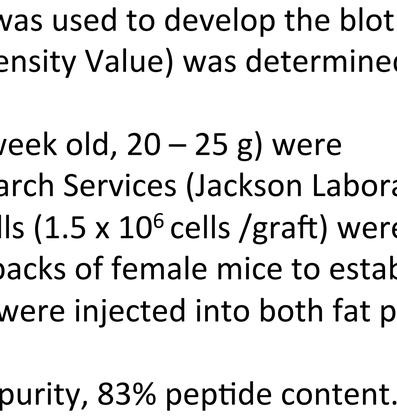
Combination Treatments: T-47D xenografted mice were treated i.p. with SOR-C13 (300 mg/kg) daily and Paclitaxel at low (6 mg/kg), medium (10 mg/kg) or high (16 mg/kg) doses weekly or a combination of the two drugs. SKOV-3 xenografted mice were treated i.p. with SOR-C13 (300 mg/kg) daily at with CAT at 20/6, 40/12 and 60/18 mg/kg weekly.

Results

TRPV6 in cells and xenografts

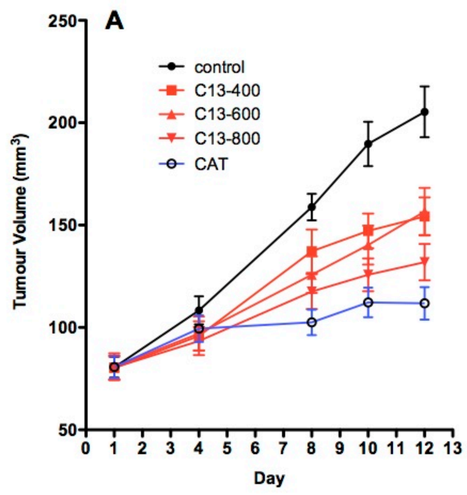


TRPV6 Western Blot of breast (T-47D) and ovarian (SKOV-3) cell line lysates. The arrows indicate the fully glycosylated TRPV6 and deglycosylated.



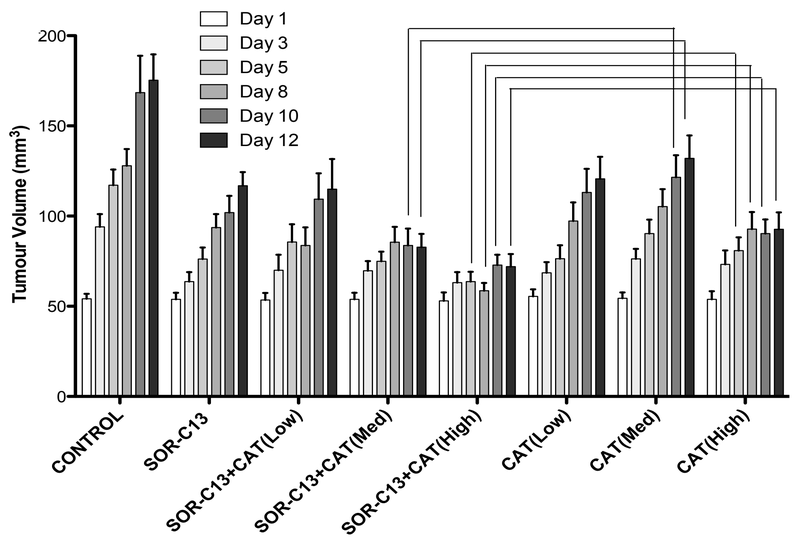
TRPV6 Western Blots of xenograft tumours derived from SKOV-3 and T-47D cells. The values are the mean ± SEM with n indicated on the graph. Error bars are above for glycosylated TRPV6 and below for deglycosylated.

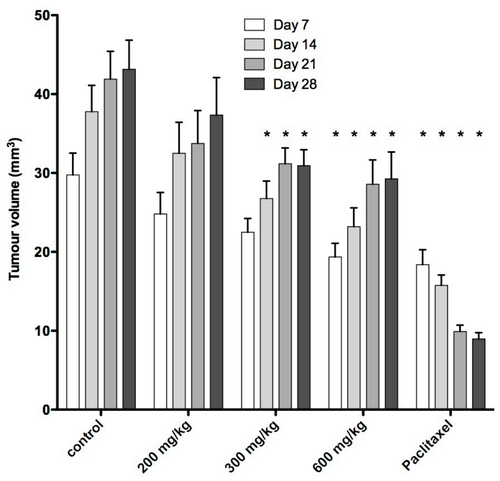
Ovarian and Breast Xenograft responses



Dose Response of ovarian cancer (SKOV-3) xenografts. Daily doses in mg/kg. Mean ± SEM, n = 24. CAT at 60/18 mg/kg

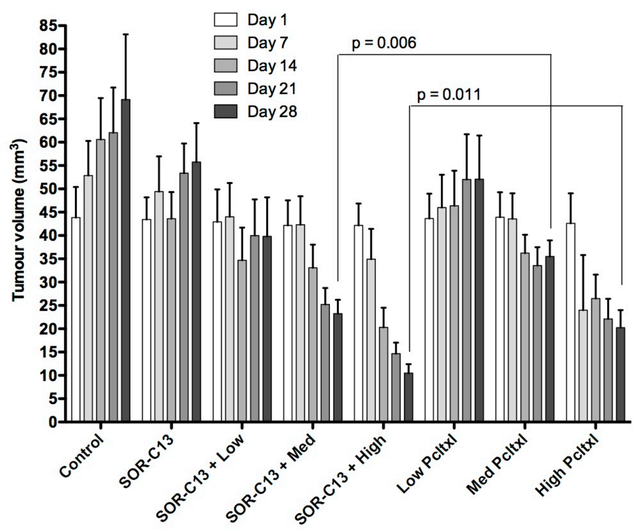
SKOV-3 Combination Study at Constant SOR-C13 (300 mg/kg) and low (20/6 mg/kg) medium (40/12 mg/kg) and high (60/18 mg/kg) CAT. Lines Connect combination treatments > CAT alone, $p<0.05$. Mean ± SEM, n = 14 – 20.





Dose response in breast (T-47D) xenografts with Daily i.p. injection. Mean ± SEM, n = 14 – 18. Paclitaxel at 16 mg/kg

T-47D combination Study at constant SOR-C13 (300 mg/kg) and low (6 mg/kg) medium (10 mg/kg)) and high (16 mg/kg) Paclitaxel. Lines connect Combination treatments > Paclitaxel $p<0.05$. Mean ± SEM, n = 14 – 20.



Summary:

- SKOV-3 and T-47D xenografts suggest a dose response.
- SKOV-3 xenografts also indicate saturation of TRPV6 'receptors' at these doses.
- SKOV-3 xenografts indicate an additive combination effect.

References:
Bowen, C.V., DeBay, D., Ewart, H.S., et al. 2013. *In vivo* Detection of Human TRPV6-Rich Tumors with Anti-Cancer Peptides Derived from Soricidin. PLOS ONE 10.1371/journal.pone.0058866
Zhuang L, Peng JB, Tou L, et al. 2002. Calcium-selective ion channel, CaT1, is apically localized in gastrointestinal tract epithelia and is aberrantly expressed in human malignancies. Lab Invest 82:1755 – 1764.

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