Validation of the transient receptor potential vanilloid 6 channel (TRPV6) as a potential biomarker in ovarian cancers

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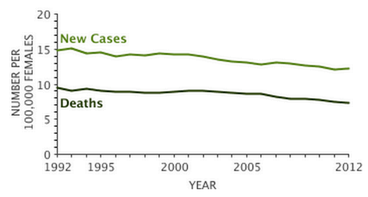
ABSTRACT: During the last 15 years over-expression of TRPV6 in multiple epithelial cancer types (breast, prostate, ovarian) has been reported and recently it has been classified as an oncochannel. Over-expression of this non-voltage gated calcium channel has been linked to oncogenesis and cancer progression in epithelial-derived cancer types. Pre-clinical data, toxicology and efficacy studies supported the clinical evaluation of the first peptide-based TRPV6 inhibitor presently in a Phase I clinical trial, confirming that the channel represents a potential 'druggable' target in oncology. While TRPV6 is recognized as an oncochannel with the potential to be used as a biomarker in oncology, there are few comprehensive quantitative data available to assess the degree of over-expression of TRPV6 in tumours. To confirm the identity of TRPV6 as a potential biomarker that can be used in smart targeted therapies or for diagnostic purpose, we present: 1) A comprehensive assessment of the mRNA expression of TRPV6 gene in 191 ovarian biopsies (26 normal and 165 malignant tissue samples) covering the 5 histological subtypes of this cancer: clear cell, endometrioid, mucinous, low grade serous and high grade serous. 2) TRPV6 protein expression assessed with immunohistochemistry in tissue microarrays for ovarian cancers (101) compared to normal (23) biopsies. In the 191 ovarian biopsies, TRPV6 mRNA ranges from 2-fold to 2074-fold larger than for normal controls in 159/165 tumours ($p < 0.0001$). Elevated TRPV6 gene expression occurred across all ovarian tumour stages, grades, histopathological types and subtypes with the mean of all carcinomas 69-fold (n = 20) and adenocarcinomas 100-fold (n = 134) above normal tissue (n = 26). Separation into five histological subtypes of ovarian cancers showed that clear cell (80-fold, $p = 0.002$), endometrioid (78-fold; $p < 0.0001$), mucinous (23-fold; $p = 0.0258$), high grade serous (88-fold; $p < 0.0001$) and low grade serous (49-fold; $p = 0.0229$) were statistically greater (Mann Whitney test) than healthy tissue. Immunohistochemistry results analysis of the ovarian biopsies was based on a ranking scale of 5 (0 to +4) for staining intensity. Of the 23 normal biopsies; 8 were at '-' ranking, 8 undecided ('-/+'), two at ++ and none above this ranking while 98/101 cancer biopsies were +1 or greater (97%) with 85 +2 or greater (84%). The ovarian cancer biopsies represented both early and late stages across the main cancer types. These data clearly support TRPV6 as a novel and significant biomarker for TRPV6-targeted treatment modalities such as TRPV6 inhibitors, peptide drug conjugates (PDCs), and tumour imaging. To confirm smart drug delivery through TRPV6 we engineered a prototype PDC of a TRPV6-binding peptide (SOR-C27) conjugated with Paclitaxel to be tested in mice xenografts of prostate cancer (PC-3). The PDC outperformed Paclitaxel alone, a mixture of Paclitaxel and peptide, and peptide alone in reducing tumour growth .

Introduction

Ovarian Cancer (from SER Fact Sheets USA)

Estimated New Cases in 2015	21,290
% of All New Cancer Cases	1.3%
Estimated Deaths in 2015	14,180
% of All Cancer Deaths	2.4%



Percent Surviving 5 Years
45.6%
2005–2011

Ovarian cancer is the most lethal gynecological malignancy. Bimanual examination, CA-125 and transvaginal ultrasonography together allows detection of only 30–45% of women with early-stage disease. There is a need for effective biomarkers both for ovarian cancer therapeutic targeting and early diagnostics. Many epithelial cancers over-express TRPV6, a non-voltage gated calcium channel (Zhuang et al., 2002) now classified as an oncochannel. This is the first comprehensive study demonstrating the association of TRPV6 expression with ovarian cancer clinical biopsies as well as the therapeutic potential of TRPV6 targeting drug delivery.

Materials & Methods

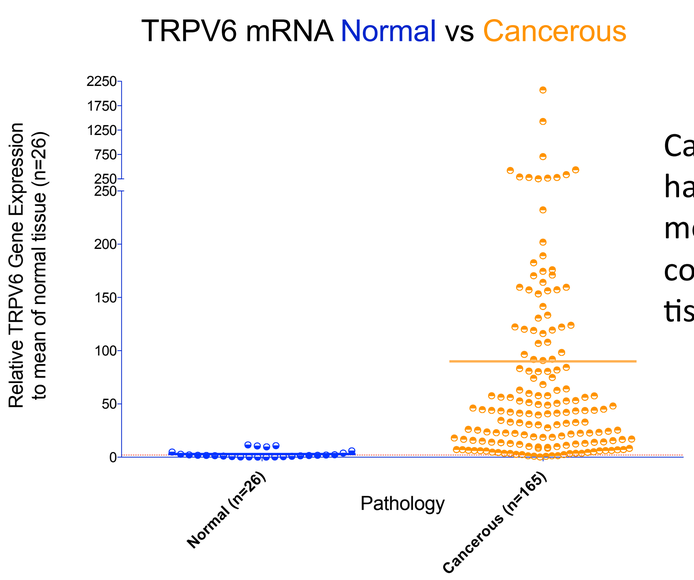
TRPV6 mRNA Expression: Human TRPV6 and β-actin mRNA levels in normal and tumour tissues were determined in 192 ovarian biopsies using three TissueScan Ovarian Cancer cDNA Arrays from OriGene Technologies, Rockville MD. Each TissueScan Ovarian Cancer Array contained cDNA prepared from 48 biopsies of ovarian normal and tumour tissue samples. For mRNA expression two-step reverse-transcriptase quantitative real time PCR (RT-qPCR) using TRPV6 TaqMan® primers and probe set (Life Technologies) were used and determination of gene expression levels was done to the specifications outlined in the MIQE guidelines (Bustin et al., 2009)

Immunohistochemistry: TRPV6 protein expression was evaluated in 123 independent biopsies of various ovarian cancers at various stages and grades using the anti-TRPV6 antibody (Santa Cruz, H-90) and a goat anti-rabbit IgG-HRP secondary antibody (Santa Cruz). A score of – (negative) to ++++ (intense staining) was used to grade TRPV6 expression.

Xenograft. PC3 tumour fragments (app. 30mm^3 fragments /graft) were implanted on the flanks (1 per mouse) of nude CD1 female mice (Jackson Laboratories) to establish the xenografts. When tumour reached a volume of 100 mm^3, i.v. treatments were initiated with the SOR-C27 paclitaxel PDC (19.5 mg/kg), SOR-C27 alone (14.9 mg/kg), SOR-C27 (14.9 mg/kg) + paclitaxel (4.3 mg/kg), paclitaxel alone (4.3 mg/kg) at D1, 4, 8, and 11. Tumours were measured 3 times a week using a digital caliper until termination.

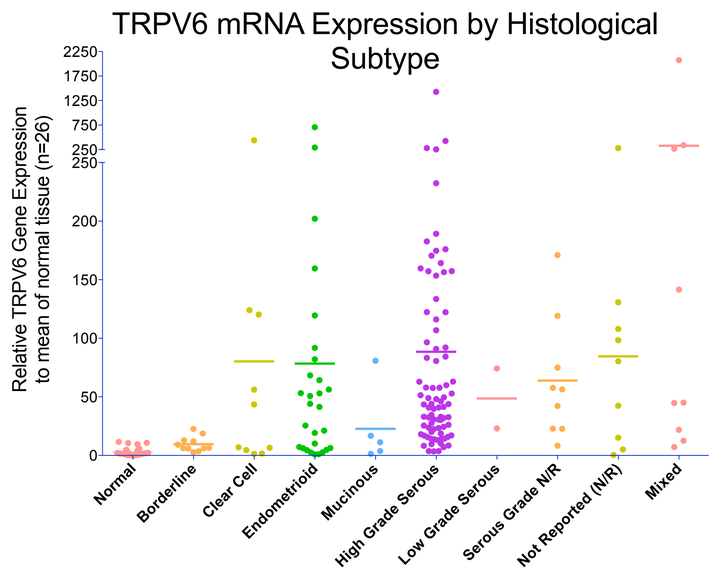
Results

TRPV6 mRNA expression in ovarian biopsies



Cancerous biopsies have 2- to 2074-fold more TRPV6 mRNA compared to normal tissues.

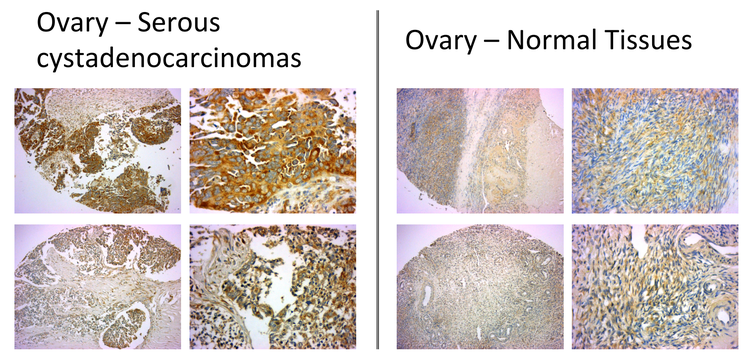
TRPV6 mRNA expression in ovarian biopsies (continued)



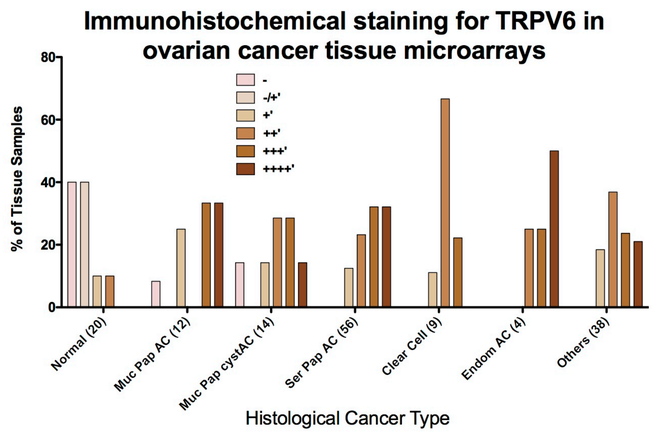
Results indicate that all ovarian cancer subtypes have significant TRPV6 mRNA over-expression vs normal ovarian tissue.

TRPV6 protein expression in ovarian biopsies

Results indicate that ovarian carcinoma tissue biopsies have a higher TRPV6 staining density

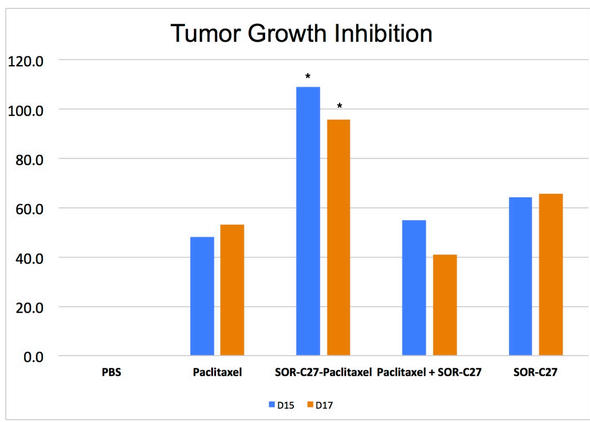


97% of tumour biopsies had a ranking of '+' or greater. 84% of samples were ranked '++' or greater. 90% of normal samples were lower than '++'.



In vivo efficacy of a TRPV6-targeting peptide paclitaxel conjugate

The TRPV6-targeting PDC SOR-C27-paclitaxel conjugate shows significantly more anti-tumour efficacy than equivalent doses of either compounds alone or administered together.



Summary:

✓ This comprehensive study of ovarian biopsies demonstrate that TRPV6 is a valid ovarian cancer biomarker for targeted therapy or diagnostics

References:

Bustin SA et al. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009 Apr;55(4):611-22.

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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