High prevalence of elevated TRPV6 mRNA in pancreatic ductal adenocarcinoma

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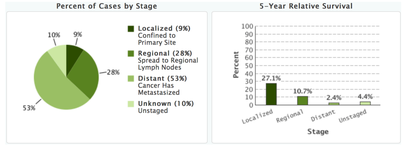
Abstract

SOR-C13, a 13-mer peptide derived from soricidin, the paralytic protein component of saliva of the Northern Short-tailed shrew is currently in an open-label, all comers Phase I Clinical Trial for the treatment of epithelial-derived cancers. SOR-C13 specifically targets and inhibits the TRPV6 calcium channel - a recognized oncochannel over-expressed in a number of epithelial cancers (*e.g.* breast, ovarian, prostate). TRPV6 over-expression is associated with a poor prognosis particularly with breast and prostate cancers. SOR-C13 is the first TRPV6-targeting drug to enter clinical development. Pancreatic patients were enrolled in the Phase I clinical trial for SOR-C13, for this reason the TRPV6 gene expression in pancreatic tumour biopsies was assessed. Pancreatic ductal adenocarcinoma represents 90% of the pancreatic tumours and is associated with a poor prognosis compared to endocrine tumours. Endocrine tumours are generally associated with a good prognosis. Association of TRPV6 oncochannel expression with aggressive pancreatic ductal carcinoma compared to endocrine tumours has never been investigated. TRPV6 gene expression was assessed in 19 pancreatic tumour biopsies including four ductal (grade I to III), one adenocarcinoma (grade II), one acinar cell (exocrine), four Islet cell (endocrine) and 9 neuroendocrine tumours by two-step RT-qPCR. TRPV6 and β-actin (reference gene) mRNA expression was evaluated in duplicate in a TRPV6 plus β-actin duplex two-step RT-qPCR assay. The adenocarcinoma, acinar cell, 75% of the islet and 67% of the neuroendocrine tumours had very little or no detectable TRPV6 mRNA expression (C_T >40). The C_T of TRPV6 mRNA expression was between 30 and 33 in 100% of the pancreatic ductal adenocarcinoma biopsies studied (mean 31.72 ± 0.94), indicating a high level of TRPV6 expression in this type of aggressive pancreatic cancer. The β-actin C_T was between 25 and 30 for all tumours (mean 28.54 ± 1.20) indicating that the lack TRPV6 amplification in a majority of the endocrine tumours was not due to RT-qPCR failure or a lack of quality of the biopsy cDNA. Results indicate that drugs specifically targeting TRPV6 oncochannel like SOR-C13 have the potential to be used to treat the high medical need in pancreatic ductal adenocarcinoma.

Introduction

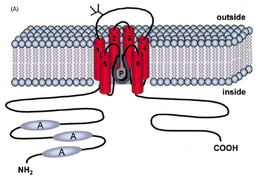
Estimated New Cases in 2015	48,960
% of All New Cancer Cases	3.0%
Estimated Deaths in 2015	40,560
% of All Cancer Deaths	6.9%

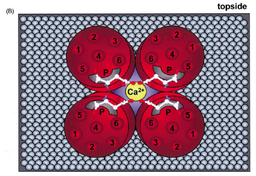


Percent Surviving 5 Years
7.2%
2005-2011

Pancreatic cancer is the 12th most common cancer in the United States, making it a relatively rare cancer. Despite the rarity of the cancer, it is the third leading cause of cancer-related death in the United States. This is due to the poor relative 5-year survival rate of pancreatic cancer, with only 7.2% of patients surviving 5 years after diagnosis. The low relative survival is in part due to the difficultly to detect early, with only 9.0% being diagnosed at early stage when the disease is localized and lack of effective treatments. It is estimated that there will be 48,960 new cases of pancreatic cancer, with an estimated 40,560 people dying of the disease in 2015 (National Cancer Institute, 2016).

TRPV6



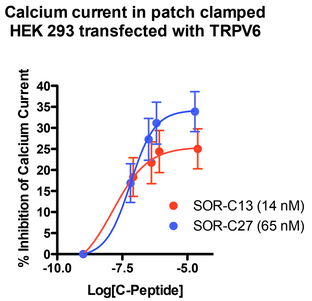


den Dekker, 2003

TRPV6 is a membrane bound channel that is highly selective for Ca^{2+} and expressed in organs to mediate calcium absorption by the intestine. TRPV6 channels regulate cancer cell proliferation, apoptosis, angiogenesis, migration and invasion during tumour progression. The increased expression of the constitutively active TRPV6 channels in the plasma membrane of cancer cells have cancer promoting effects by enhancing Ca^{2+}-dependent proliferative response, metastasis as well as resistance to apoptotic-induced cell death. TRPV6, a recognized oncochannel over-expressed in a number of epithelial cancers has been found up-regulated in breast, prostate, ovarian, thyroid and colon cancers with respect to normal controls.

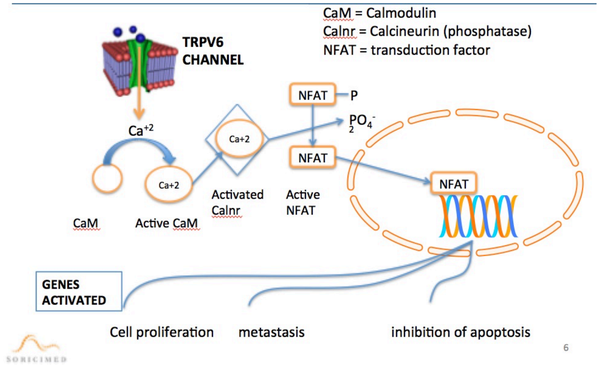
References:
Bowen, C.V., DeBay, D., Ewart, H.S., et al. 2013. *In vivo* Detection of Human TRPV6-Rich Tumors with Anti-Cancer Peptides Derived from Soricidin. PLOS ONE 10.1371/journal.pone.0058866
Bustin SA, Benes, V., Garson, J.A., Lellemans, J., Huggett, J., Kubista, M., Mueller, R., Notan, T., Pfaffl, M. W., Shipley, B.L., Vandescompele, J. and Wittwer, C. T. 2009. The MIQE guidelines: Minimal information for publication of quantitative real-time PCR experiments. Clin. Chem. 55: 611 – 622.
den Dekker et al. 2003. The epithelial calcium channels, TRPV5 & TRPV6: from identification towards regulation. Cell Calcium, 35: 497-507.

TRPV6 Inhibition by Soricidin Peptides



Two novel peptides have been developed at Soricimed that bind to and inhibit TRPV6 calcium channel activity. The peptides, SOR-C13 and SOR-C27, derived from soricidin, are selective, high-affinity antagonists of human TRPV6 channels having an IC_{50} (K_d) 14 ± 1.3 nM and 65 ± 1 nM, respectively (Bowen et al. 2013).

TRPV6 Mechanism of Action

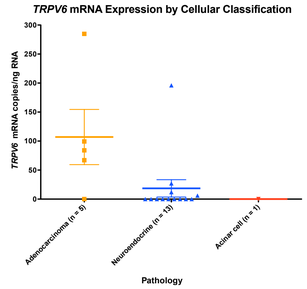


Materials and Methods

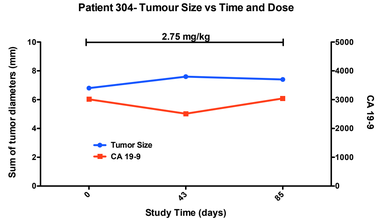
TRPV6 mRNA Expression: Human TRPV6 and β-actin mRNA levels in tumour tissues were determined in 19 pancreatic biopsies using a TissueScan Pancreatic Cancer qPCR Array (OriGene Technologies). For mRNA expression, two-step reverse-transcriptase quantitative PCR (RT-qPCR) using TRPV6 TaqMan® primers and probe sets (Life Technologies) were used. Determination of gene expression levels was done to the specifications outlined in the MIQE guidelines (Bustin et al., 2009). A TRPV6 RNA standard curve was prepared for absolute quantification of the TRPV6 mRNA in each pancreatic biopsy.

SOR-C13 Phase I Clinical: Soricimed, was an open-label, all comers, dose escalation study (3x3) to assess safety and tolerability of SOR-C13 in subjects with advanced solid cancers commonly known to express TRPV6. SOR-C13 is a first-in-class drug candidate that specifically targets and inhibits the TRPV6 calcium channel. Subjects received intravenous infusion of SOR-C13 over 90 minutes for 3d on/4d off, 3d on/11d off cycle (21d per cycle). Two subjects with advanced pancreatic adenocarcinoma were enrolled in the Phase I clinical study. Both subjects failed at least three prior regimens of anticancer therapy.

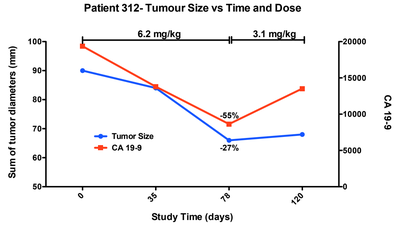
Results



TRPV6 mRNA expression analysis of the pancreatic cancer biopsies showed a significant difference (p = 0.0304) in the mean of *TRPV6* mRNA copies/ng RNA in 5 adenocarcinoma (107 ± 48) and the 13 neuroendocrine (19 ± 15). The one acinar cell biopsy had 0 copies/ng of TRPV6.



Subject 304 had stable disease over 4 cycles (12 weeks) of SOR-C13 treatment at a dose of 2.75 mg/kg. The level of the CA 19-9 pancreatic tumour biomarker returned to baseline after an initial 16% reduction after 2 cycles of treatment.



Subject 312 (KRAS mutation) had a 27% reduction in tumour size after 4 cycles of treatment at 6.2 mg/kg of SOR-C13. The reduction in tumour size corresponded with a 55% decrease in CA 19-9. A dose dependent effect was observed when the tumour started to grow after dose reduction to 3.1 mg/kg for cycle 3 because of pneumonia (not a drug related DLT).

Summary:

- The *TRPV6* expression for pancreatic adenocarcinoma biopsies ranged between 67 and 285 TRPV6 copies/ng RNA in 4/5 biopsies, indicating a high prevalence of TRPV6 expression in this type of aggressive pancreatic cancer compared to neuroendocrine tumours which have very little or no detectable TRPV6 mRNA expression (<30 TRPV6 copies/ng RNA) in 12/13 biopsies analyzed.
- *TRPV6* may be a key oncogene in pancreatic adenocarcinoma tumours, identifying them as possible targets for SOR-C13 treatment.
- Promising evidence of activity of SOR-C13 against advanced pancreatic adenocarcinoma was observed, with stable disease lasting 12 weeks (subject 304) and 23 weeks (subject 312).
- SOR-C13 treatment at 6.2 mg/kg significantly reduced the pancreatic tumour size, which correlated with a reduction in CA 19-9 in subject 312.