Molecular profiling of hormone-resistant prostate cancer cells with TRPV6 ancochael knockout/knockdown

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Abstract

TRPV6 channel knockdown is a recognized cannabinoid receptor expressed in a number of epithelial cancers (e.g. breast, ovary, prostate). TRPV6 is a member of a large subfamily of ion channels responsible for the modulation of cell proliferation and apoptosis. In prostate cancer, TRPV6 has been shown to play a role in tumor proliferation and survival. In this study, we used a combination of CRISPR-Cas9 and transient expression to create a prostate cancer cell line that lacks TRPV6 expression. The impact of TRPV6 knockdown on cell proliferation, apoptosis, and migration was assessed using cell-based assays. Our results indicate that TRPV6 knockdown results in decreased cell proliferation, increased apoptosis, and decreased migration in prostate cancer cells. These findings suggest that TRPV6 may be a potential target for the development of novel therapeutic strategies for prostate cancer.

Materials & Methods

Cell Culture and TRPV6 knockdown by shRNA

PC-3 cells were obtained from ATCC and cultured as recommended. Cells were transfected with either TRPV6 shRNA 1, shRNA 2, or shRNA 3 from Biovector using Lipofectamine® RNAiMax. Cells were harvested at 80-100% confluence 72 hours after transfection.

Generation of TRPV6 Knockout Cell Lines

Two TRPV6 knockdown cell lines (PC-3 TRPV6-1B and PC-3 TRPV6-2B) were generated using Ge-neEff™ CRISPR Cas9 vectors in conjunction with CRISPR Designer software. The TRPV6 gene was targeted using two sgRNAs (sgRNA 1 and sgRNA 2) to generate a frameshift mutation in the TRPV6 coding sequence. Two CRISPR-Cas9 vectors were introduced into PC-3 cells with caspase-3 expressing pBR-322 plasmid. The colony formation assay was performed to identify colonies with increased Cas9 expression and a frameshift mutation in the TRPV6 coding sequence. The colonies were transfected with TRPV6 shRNA plasmids using Lipofectamine® RNAiMax. The cells were then analyzed using qRT-PCR and western blot to confirm the knockdown of TRPV6 expression.

Results

Analysis of 127 genes by RT-qPCR TaqMan® Array

The Venn Diagrams depict the number of differentially expressed genes in each TRPV6 treatment. PC-3 TRPV6 knockdown TRPV6-1B (n = 3), PC-3 TRPV6 knockdown TRPV6-2B (n = 3) and untreated PC-3 cells (n = 3) were analyzed using the TaqMan® Array. The knockdown of TRPV6 resulted in a decrease in the expression of many genes involved in prostate cancer progression.

Down-Regulated Genes

There were 26 genes down-regulated in TRPV6-1B, 27 in TRPV6-2B knockdown, 9 in shRNA 3 knockdown. There were significantly more genes up-regulated in TRPV6-1B and TRPV6-2B knockdown, compared to untreated PC-3 cells, indicating a role for TRPV6 in prostate cancer progression.

Up-Regulated Genes

There were 10 genes up-regulated in TRPV6-1B and 17 up-regulated in TRPV6-2B knockdown, compared to untreated PC-3 cells. These results suggest that TRPV6 knockdown may be a potential target for the development of new therapeutic strategies for prostate cancer.

Summary

Trichloroleptin (TRPV6) plays a role in prostate cancer progression. The analysis of differentially expressed genes in the TRPV6 knockdown cell lines revealed a decrease in the expression of many genes involved in prostate cancer progression. These findings suggest that TRPV6 may be a potential target for the development of novel therapeutic strategies for prostate cancer.