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Targeted Depletion of VR1 in Murine Nociception Models for Treatment of Analgesics Resistant Pain

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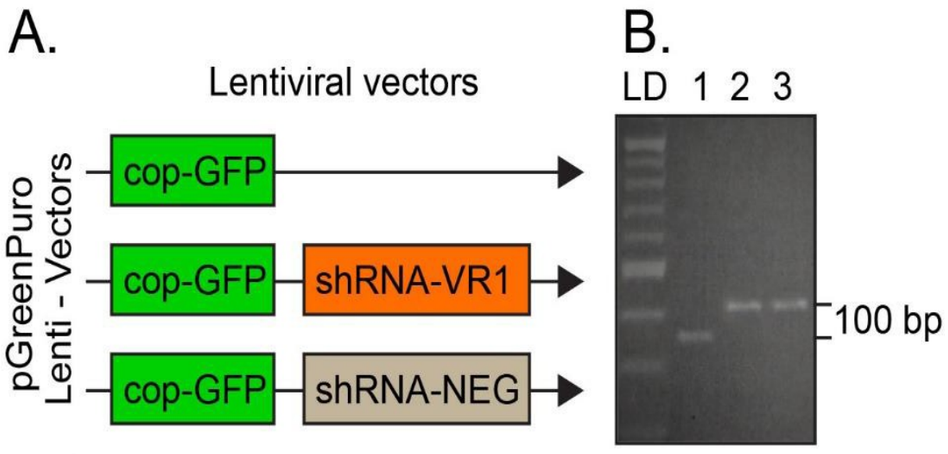
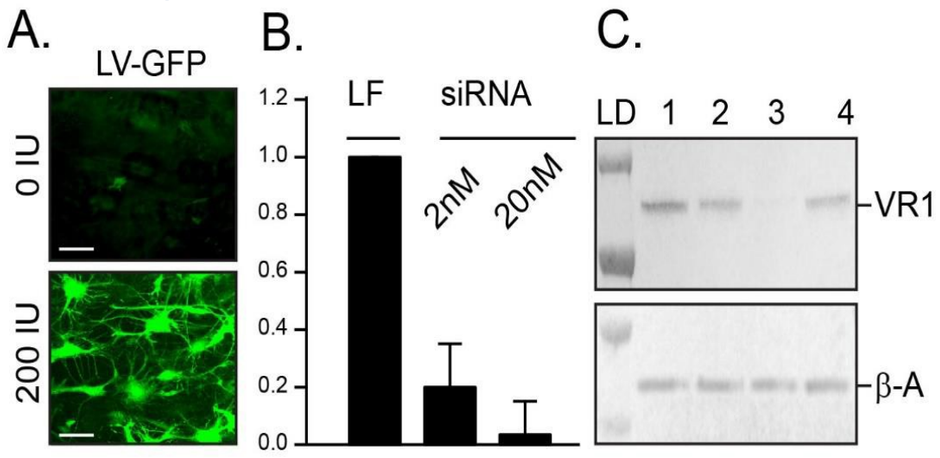
Introduction: Trigeminal neuralgia (TGN) is characterized by severe episodes of facial pain due to persistent activation of one or more branches of the trigeminal nerve. The transient receptor potential (TRP) ion channel receptor TRPV1 (also known as Capsaicin Receptor, Vanilloid Receptor 1 or VR1) is known to induce burning pain by depolarizing a subsets of a special type of sensory neurons called nociceptors (e.g., C and type 2 A-δ nociceptors) and plays a key role in the molecular mechanism. The majority of currently used analgesic drugs for trigeminal neuralgia (TGN) have a limited safety margin and have adverse effects. Thus, there is a pressing need for novel and effective means for better management of facial pain, with the ideal analgesic treatment anticipated to show high selectivity, tolerability and long-acting effects localized to the site of distress.



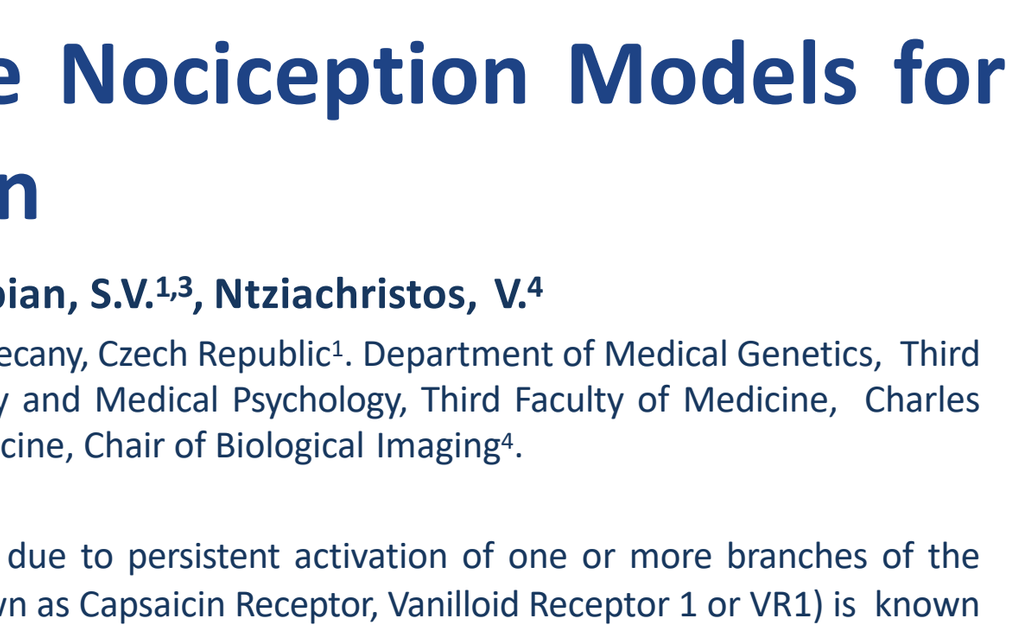
Result 1: Design of non-replicating LV vectors for silencing pain sensing nociceptors with production of vectors: **(A)** GFP expressing LV vector (cop-GFP), GFP and shRNA-VR1 interference encoding LV vector (cop-GFP and shRNA-VR1) and GFP and scrambled shRNA-VR1 encoding LV vector (cop-GFP and shRNA- NEG (negative control). **(B)** Electrophoretic gel demonstrating successful design and production of the vectors of interest used in this study. Presence of shRNA encoding inserts evident via 100bp differential band mobility.



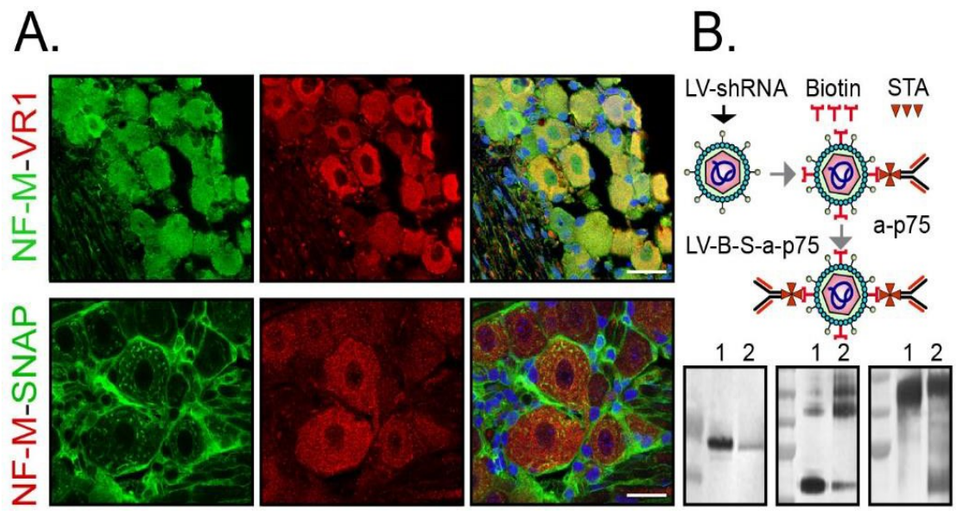
Result 2: **(A)** Verification of the viability and infectivity of in-house made LV expressing reporter cop-GFRP in mouse cortical cultures (21 days old) (IU infectious units) **(B)** Demonstration of the elimination of the VR1 transcripts in cultured cortical neurons *in vitro* after exposure to siRNA and relative QPCR assessment with GAPDH endogenous control normalisation **(C)** inhibition of the expression of the VR1 expression in primary neuronal cultures using WB analysis with the use of various siRNA concentrations.

Aims of the project

This project aims to validate the depletion of VR1 trigeminal nerve nociceptors to alleviate drug-resistant cancer and neuropathic pain. It will test a LV based gene therapy based methodology invented by the Czech PI, which has passed the initial tests in alleviating the pain response in murine models. This project will extend the gene therapy tests on drug-induced and cancer pain models. Along with assessing the nociceptive response in behavioural tests of mice, a range of molecular readouts such as expression of mGluR5 and pain-responsive ERK1/2, Arc/Arg3.1 and c-*fos* will be quantified using biochemical and fluorescence means. Tests of the pain killing effects of LV gene theapy vectors will be extended also to cancer pain using xenografts with RSOM imaging for visualizing the skin neuro-vascular response, and MSOT for mapping pain sensation with brain imaging in living animals *in vivo*.



Result 3: **(A)** Schematic of the rodent TG sensory system with afferent and primary plus secondary sensory neurons in the TGG and brain stem sensory nuclei (left). Primary sensory axons in red, secondary projections in blue. TG-trigeminal ganglion. TGG – retroaxonally labeled TG nociceptors with mu75 IgG *in vivo*. **(B)** Immuno-fluorescence images of retrogradely labeled neurons in TGG (left). VR1 and p75NTR positive nociceptors in TG ganglion *ex vivo*.



Result 4: **(A)** Immuno-fluorescence confocal images of neurons in TGG labeled with anti-VR1 nociceptor antibody and neurofilament M neuronal marker (top) and SNAP-25 neuronal SNARE protein. Note selectivel enrichment of TGG nociceptors with the VR1 channel complex. **(B)** Schematic illustration of sILVER therapeutic vector with biotinylation (Biotin) and targeting with the use of streptavidin (STA) coated anti-p75NTR antibody. Biochemical verification of the material using anti p-24, -VSVG and -p75 antibodies (left-right).

Methods

Commercially available plasmids were used for LV production. These included the pMD2.G encoding VSVG, pWPT–copGFP for transgene expression and pCMV–d R8.2 for packaging. These plasmids were bacterially amplified followed by purification and extraction. Stable transfection of HEK293FT cells was used for LV production under level II safety containment. The pellet containing the LV was re-suspended in PBS (50–100 µl) and stored at –80°C and applied to neuronal cultures. For immunofluorescence confocal imaging, samples were stained as 30 um free-floating TGG sections, which were incubated with primary antibodies diluted in 0.1 M PBS containing 0.3% Triton-X 100 overnight at 4 °C, followed by development using fluor labeled secondary antibodies. After mounting and covering, samples were imaged with a Zeiss Axio Imager Z1 fluorescent microscope (Carl Zeiss, Jena, Germany) or Leica TCS SP8X confocal microscope (Leica Microsystems Mannheim, Germany). For confocal imaging, sections were excited with appropriate excitation lines and filters, with fluorescence signal collected and analyzed using in AxioVision Rel 4.8 software (Carl Zeiss), LAS AF software, and ImageJ 1.47 software (NIH). Image brightness and contrast have been adjusted in a standardized manner for all of the images. All images and graphs were generated and assembled in figures using Igor Pro and Adobe Illustrator (Adobe Systems).

Future work: The follow up steps are (1) validation of innovative therapeutic vectors in a capsacin-incuced pain model of mice, (2) optimization of the head and neck cancer pain model using xenografts with (3) targeted elimination of cancer pain with novel therapeutic sILVER with histochemical validation via nociceptive markers and (4) functional validation using RSOM and MSOT imaging in living animals *in vivo*.

Bayerische Staatskanzlei

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