TRPM8-mediated response to cold in human DRG neurons and modulation by the chemotherapy agent oxaliplatin.

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Introduction

Chemotherapy-induced peripheral neuropathy (CIPN), is a serious dose-limiting adverse effect induced by several chemotherapy agents and can lead to dose reduction or discontinuation of therapy. Manifestations of CIPN include: mechanical and cold allodynia, burning pain and tingling numbness. Following the cessation of chemotherapy, gradual recovery from peripheral nerve damage occurs in most patients but, in a significant number of cases, pain may persist. Cold allodynia is a very common instance with CIPN and growing efforts have been focused on the identification and development of pharmacological agents that can treat cold-related pain in CIPN patients. The mediators of cold pain sensation in human remain poorly characterized, although several ion channel candidates have been proposed. Transient Receptor Potential Cation Channel, Subfamily M, Member 8 (TRPM8) is considered a prime candidate for the transduction of low temperature-induced pain. Thus, it is a potentially valuable target for the development of novel agents with the potential to treat cold allodynia.

Methods

Human DRGs were collected within 2 hrs. post-mortem and immediately transferred to AnaBios' cold plegic solution to preserve viability. Cells were enzymatically dissociated and plated on PDL-coated glass coverslips.

Immunohistochemistry. TRPM8 immunoreactivity was demonstrated in paraformaldehyde fixed, frozen sections of human DRG using polyclonal anti-TRPM8 (Alomone ACC-049, peptides 917-929, 3rd extracellular loop) and Dako Envision Flexplus on a Dako Autostainer.

In situ hybridization. TRPM8 mRNA expression was demonstrated in frozen sections of human DRG, using automated ViewRNA (Affymetrix) in situ hybridization running on a Leica Bond.

qRT-PCR. qRT-PCR was used to simultaneously determine the expression of the TRPM8 and GAPDH mRNA using cDNA prepared from the total RNA samples.

Calcium imaging. Cells were loaded with Fluo-8-AM and time-lapse images (485 nm Ex and 530 nm Em) were acquired.

Results

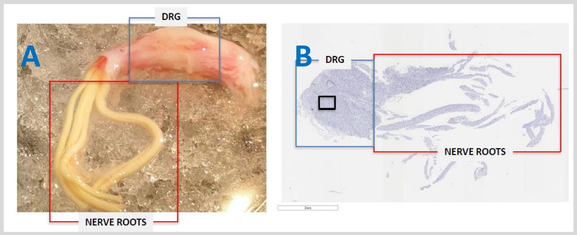


Figure 1. Human Dorsal Root Ganglion.
A. Fresh DRG and efferent nerve fibers dissected out from health human donor. **B**. haematoxylin stained human DRG section.

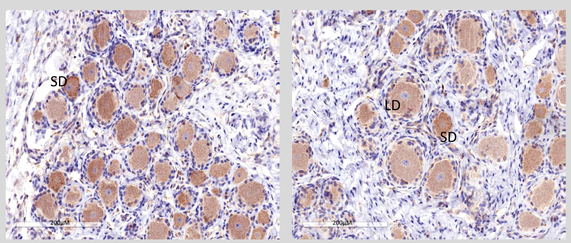


Figure 2. TRPM8-immunoactivity in Large (LD) and Small (SD) Diameter Human DRG Neurons.

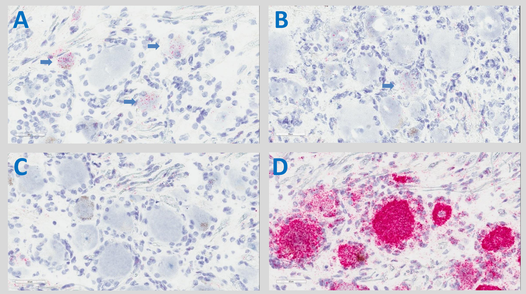
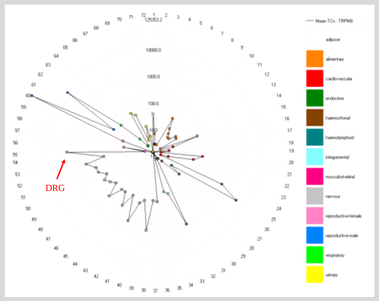


Figure 3. Expression of TRPM8 mRNA in Human DRGs.
A and B. Expression of TRPM8 mRNA. **C**. Staining of negative control probe to bacterial RNA, DapB. **D**. Staining of probes for housing keeping genes (bACTIN, GAPDH and PPIB).



Tissue with copy numbers for TRPM8 > 1000:

- Prostate (#60)
- Testis (#62)
- Pituitary gland (#25)
- DRG (#55)

Figure 4. TRPM8 Gene Expression. The TRPM8 mRNA in human tissues are arranged as a stellar-plot. The mean copy number for each of 72 different human tissues, from three (non-matched) donors - are shown as spokes on a wheel.

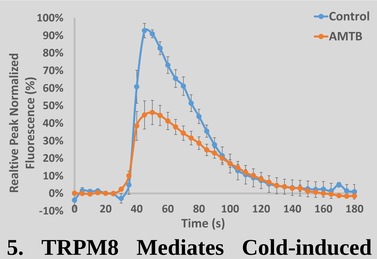


Figure 5. TRPM8 Mediates Cold-induced Response in Human DRG Neurons. Cold-induced activation of human DRG neurons and response inhibition by the TRPM8 blocker AMTB (20 μM).

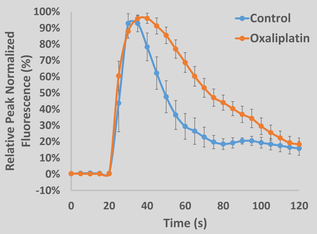


Figure 5. Oxaliplatin Enhances Cold-induced Response in Human DRG Neurons. The kinetics of the cold-induced response was prolonged following oxaliplatin treatment (See also table below).

Table 1. Kinetic Change of Cold-induced Response.

Condition / Relative decay timepoint (sec)	30%	50%	70%
Control	15 ± 3	27 ± 4	49 ± 4
Oxaliplatin	26 ± 2	44 ± 4	61 ± 4
% of increase	70%	61%	25%

Table 2. Percentage of Cold-activated Cells.

	Total	Cold Responsive	% of Responsive Cell
Control	639	128	20 ± 2%
Oxaliplatin	494	118	24 ± 4%

Conclusion

A subset of human smaller diameter DRG neurons express TRPM8, which can be detected both by mRNA hybridization as well as immunostaining. In addition TRPM8-mediated response to cold buffer can be recorded in human DRG neurons. This response is markedly but not completely inhibited by TRPM8 blocker AMTB.

Pre-treatment with chemotherapy drug oxaliplatin induced significant kinetics change of cold-induced response, supporting the notion that sensitization of TRPM8 may significantly contribute to the cold allodynia which is frequently reported by patients treated with oxaliplatin.