HSV Vectors - Approaches to the Treatment of Chronic Pain
Prof. Joseph C. Glorioso

Gene therapy for chronic pain

- Pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”
- Nociception is the sensory component of pain. Pain perception is a cognitive function
- Chronic pain represents a pathologic state of peripheral nerves (due to injury, inflammation, arthritis, cancer, or neuropathy).
  Affects Americans more than diabetes, heart disease and cancer combined (76.2 million people affected by chronic pain ~25% of Americans)

The pain pathway

- Free nerve endings detect painful stimuli
- Primary afferents
- Synapse in Spinal SDH
- 2nd order neuron
- Synapse in Thalamus
- 3rd order neuron
- Cortex

Julius and Basbaum, Nature, 2001
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Why HSV gene therapy for pain

1. Drugs are systemic, have side effects and lose efficacy. No long-term, effective therapy for any chronic pain.
2. Non-replicating HSV vectors can persist in sensory ganglia and serve as a platform for exclusive expression of gene products that control pain signaling at the site where it arises.

HSV is a neurotropic virus ideal for gene delivery to PNS

Wt HSV infection
- Defective HSV vector infection
- LAT mRNA 2.6 kb stable intron
- Rolling strand DNA synthesis
- α (IE), β (E), γ (L) x
- CMV-EGFP IF
- Wt HSV infection
- Defective HSV vector infection
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- α (IE), β (E), γ (L) x
- CMV-EGFP IF

Criteria for vector engineering and therapeutic gene delivery to the peripheral nervous system

- Design of non-cytotoxic, non-replicating HSV platform
- Vector genome must persist as an intranuclear episome without the possibility of virus reactivation but capable of long-term transgene expression
- Localized or targeted delivery methods:
  - Dermatome inoculation
  - Vector retargeting
  - Use of specific promoter-enhancers alone or in combination with cellular microRNA binding sites incorporated into the transgene mRNA that together regulate translation.

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Cascade regulation of HSV gene expression is key to vector design

Latest generation of HSV vectors achieves long-term transgene expression in multiple cell types

Non-toxic vector with persistent expression in neurons

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Gene therapy approaches to treat chronic pain

- Interrupt pain signaling from sensory neurons (produce Enk, GABA, nerve silencing)
- Block conditions that induce hypersensitivity (block or alter ion channel functions, signaling pathways, antagonize inflammatory cytokines)
- Correct underlying mechanism for hypersensitivity to painful stimuli (reverse hyper-responsiveness)

Pain vector constructs

<table>
<thead>
<tr>
<th>Vector</th>
<th>Therapeutic gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NPE2</td>
<td>1. Preproenkephalin</td>
</tr>
<tr>
<td>2. NPEND</td>
<td>2. Endomorphin</td>
</tr>
<tr>
<td>3. NPGAD67/65</td>
<td>3. Glutamic Acid Decarboxylase</td>
</tr>
<tr>
<td>4. NPLI4</td>
<td>4. Interleukin-4</td>
</tr>
<tr>
<td>5. NPTNFRS</td>
<td>5. Tumor Necrosis Factor sol. Rec.</td>
</tr>
<tr>
<td>7. NPROPKC</td>
<td>7. Dom. Neg. PKCε</td>
</tr>
<tr>
<td>8. TRPV1</td>
<td>8. Poreless TRPV1</td>
</tr>
<tr>
<td>9. PP1</td>
<td>9. PP1α</td>
</tr>
</tbody>
</table>

Treating pain with genes whose products are opiate peptides

Preproenkephalin (PPE) is a precursor protein that is proteolytically processed to form leu- and met-enkephalin opioid peptides that inhibit neurotransmitters of pain signal transmission in the spinal cord to neurons in the brain.
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Treating chronic pain at its source

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Spinal nerve ligation model of neuropathic pain

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Neuropathic pain testing

Mechanical allodynia
  • Use calibrated von Frey filaments
  • Press against plantar surface of paw
  • Determine 50% g threshold using the up-down method
Neuropathic pain: L5 spinal nerve ligation causes mechanical allodynia

Neuropathic pain: Transduction of lumbar DRG with the PE-HSV vector reverses nerve injury-induced mechanical allodynia (3.5 wks post-op)

SHPE continues to provide an antinociceptive effect after 2 weeks of morphine treatment (tolerance)
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Osteolytic sarcoma studies

Day 1: Inject 10⁷ NCTC-2972 osteolytic sarcoma cells into medullary space of femur
Day 7: Inject 10⁷ vector subcutaneous into foot
Day 14 & 21: Perform behavioral tests and take X-rays
Day 23: Euthanize and collect s.c. for anatomical studies

Vector SHPE produces analgesia in the osteolytic sarcoma model

Ambulatory pain score

- SHZ
- SHPE

Control

SHPE

SHZ

SHPE

Vector treatment does not alter bone loss following tumor implantation

Bone destruction in osteolytic sarcoma

Control SHZ SHPE

Bone destruction in SHPE + IT NTX

0 0.5 1.0 1.5 2.0 2.5 3.0

Ambulatory pain score

14 days 21 days

* p < 0.05 vs. SHZ no tumor
* p < 0.05 vs. SHZ with tumor

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Phase I clinical trial in terminal cancer patients

Inclusion criteria
• Histologically confirmed malignancy
• Pain consistently ≥ 4 on a 10 point scale
• 200 mg/day oral morphine or equivalent

Protocol
• Dose escalation
  – 3 cohorts: $10^7$, $10^8$ and $10^9$ pfu

Phase I trial: outcome measures

• Vector inoculated intradermally in ten 100 µl injections in the dermatome(s) corresponding to the radicular distribution of the pain

Primary outcome
• Adverse Events according to NCI Common Terminology Criteria for Adverse Events (CTCAE Version 3.0)

Secondary outcomes
• Pain rating
  – Numeric Rating Scale (NRS) evaluation of pain
  – McGill Short-Form Pain Questionnaire
  – Eastern Cooperative Oncology Group Performance Status
  – SF-12 Health Survey

Diary of analgesic use daily for 30 days after vector injection

Enkephalin gene therapy reduces pain in cancer patients
Limitations of our current pain gene therapy approach

- Enkephalin therapy is promising but the treatment is transient, requiring repeat dosing.
- Long-term therapy can be achieved using neuronal cellular promoters but continuous treatment may engender tolerance and other unwanted side effects.
- Long-term therapy cannot be turned off so that therapy would continue in cases where it may not be needed.
- Solution will require regulated therapy.

Glycine receptor activation silences neuronal activity

- Ligand-gated Cl\(^{-}\) channel
- Alpha subunit alone will form functional channel
- Inhibitory
- Brain and spinal cord. Not in peripheral neurons

- Express the GlyRa subunit in primary nociceptors using a herpes simplex virus-based vector.
- Activate by exogenously applied glycine. Regulated system
- GlyR activation results in nerve silencing.

Effect of glycine on nociceptive behavior

4 weeks 30 15 0 15 (min)

Bladder wall injection

- Glycine i.p. (0-400mg/kg)
- Water loading (30mg/kg)

E1G6: Control

GlyR: Glycine receptor

20µl, 3x10\(^{10}\) pfu/ml

3µM resiniferatoxin (RTx) into the bladder and rats were placed into the metabolic cage for behavior test that included rapid bladder contractions and nociceptive behavior which was counted every 5 sec for 15 min.

Licking Freezing
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Intravenous administration of glycine (Cystitis model)

- After RTx infusion (Cystitis model), ICI was increased in E1G6 and GlyR injected rats
- ICI was significantly reduced after 1.0 mg/kg glycine i.v. Only in GlyR vector-injected rats

Glycine receptor α

- Mutations (F207A/A288G) in the GlyR cause complete resistance to activation by glycine
- This mutant GlyR can be activated by Ivermectin (treats helminth infections)

Ivermectin but not glycine activates GlyR-MUT vector in thermal hyperalgesia pain behavior
Summary

- Exploitation of the GlyR is attractive as a powerful, regulated system to block pain signaling through nociceptor silencing
- The GlyR can be readily adapted for use with anesthetics or Ivermectin
- Designs for targeted expression of the GlyR in selected subpopulations of nociceptors to define their role in chronic pain and limit therapy to the appropriate pain signaling neurons

Selective vector infection and transgene expression

- Direct Injection: skin containing nerve terminals
- Transcription: promoter/enhancer selection or cellular microRNAs altered in nociceptor neurons
- Transduction: modification of envelope for targeted receptor recognition

Targeting transgene expression with promoters of neuronal population marker genes

- NF200
- NPY (induced in Aβ after nerve injury)
- TRPV1
- CGRP
- IB4
- NGF-TrkA
- GDNF-GFRα1
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Vector design

Gene expression constructs

Promoter-regulated expression in cultured embryonic DRG cells \textit{in vitro}

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Promoter specificity in cultured embryonic DRG cells in vitro

Comparison of LAP-, CMV- and CGRP reporter gene expression

CGRP-targeting: mCherry, CGRP

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Summary

1. Gene therapy for treatment of chronic pain is feasible and effective in pain models. Human trials are needed to validate efficacy.

2. Regulated gene silencing (e.g. GlyRm) should prove effective for long-term therapy but will require targeted gene expression to prevent off-target activity with enhanced therapeutic efficacy.

3. Advanced generation vectors should provide robust long-term transgene expression.

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