## Objective
- The exact mechanisms of peripheral sensitization in the context of perineural invasion are still poorly understood.
- A critical understanding of how early sensitization occurs represents a promising strategy for prevention, drug development, and treatment of cancer pain.

## Introduction
- Pain in patients with cancer constitutes the most prevalent symptom, accounting for significant deterioration in their quality of life.
- Cancer pain is viewed as a process orchestrated by the release of pro-nociceptive molecules and the invasion of neural structures, referred to as perineural invasion.
- Early in tumor development, the release of pro-inflammatory molecules leads to the activation of receptors located on sensory neurons and surrounding support cells, promoting the sensitization of nociceptors, which transmit pain to the central nervous system.

## Methods
### Animals
Male Sprague-Dawley rats housed in temperature- and light-controlled conditions with food and water available ad libitum were used.

### Cell Line
The human HNSCC cell line FaDu was used.

### Co-culture Procedure
We have developed an in vitro model in which cancer cells are co-cultured with dorsal root ganglion (DRG) neurons, enabling us to study changes in neuronal activity that result from being in close proximity to—but not in direct contact with—FaDu cancer cells.

### Chemiluminescence Assay
Human Neuro Discovery Antibody Array C2 was used to detect 30 human cytokines, including IL-6. Membranes were imaged by using Image Quant LAS 4000 Mini and cytokine spots in the membranes were quantified using ImageJ protein analyzer software.

### Electrophysiology
Whole cell patch recording was performed to measure the electrical membrane properties of dissociated DRG sensory neurons. Glass coverslips were lifted and were transferred to a recording chamber placed on a microscope and perfused with oxygenated ACSF at room temperature. Whole cell recordings were completed within 20-28 hours after plating.

## Results
- Co-cultured neurons from older adult male rats demonstrated spontaneous activity (SA) and depolarizing spontaneous fluctuations (DSFs) more frequently than media only control neurons. Increased spontaneous activity and large (>5mV) DSFs indicate neuronal sensitization, which could indicate enhanced nociceptive activity following exposure to FaDu cancer cells in vitro.

## Conclusion
- Media conditioned by FaDu cancer cells contains many pro-inflammatory cytokines, chemokines, and growth factors known to sensitize neurons.
- Media collected after co-culture with FaDu and DRG neurons showed elevated levels of IL-6 (cytokine), which can directly induce spontaneous activity in vitro.
- Co-culture with FaDu cancer cells sensitized rat DRG neurons, with more robust effects seen in older adult rats.
- Neuronal hyperexcitability was characterized by lower current thresholds, large DSFs, spontaneous activity, and increased responses to current stimulation.
- Neuronal sensitization and mechanical allodynia were observed following treatment with exosomes released by FaDu cancer cells.
- Further studies will advance understanding of the mechanisms of peripheral sensitization and treatment and prevention of pain in patients with cancer.

## References