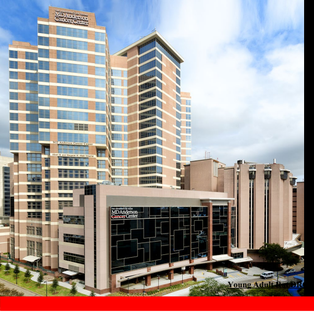

# FaDu Human Squamous Cell Carcinoma Induces Hyperexcitability of Primary Sensory Neurons

Hannah Wani, Megan L. Uhelski, Aysegul Gorur, Yan Li, Patrick M. Dougherty, Juan P. Cata

The University of Texas MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

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## 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.

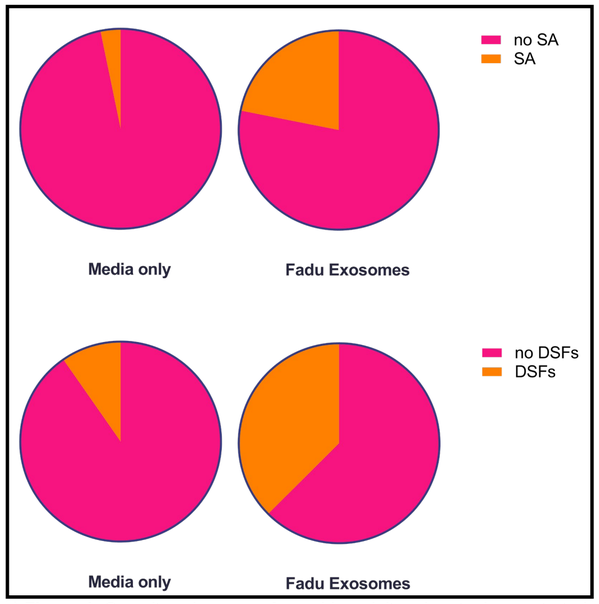
### Chemiluminescence Assay

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.

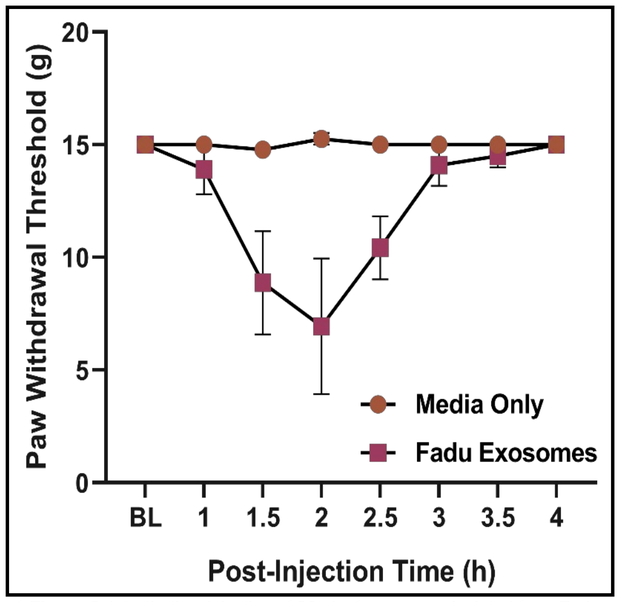
### Electrophysiology

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.

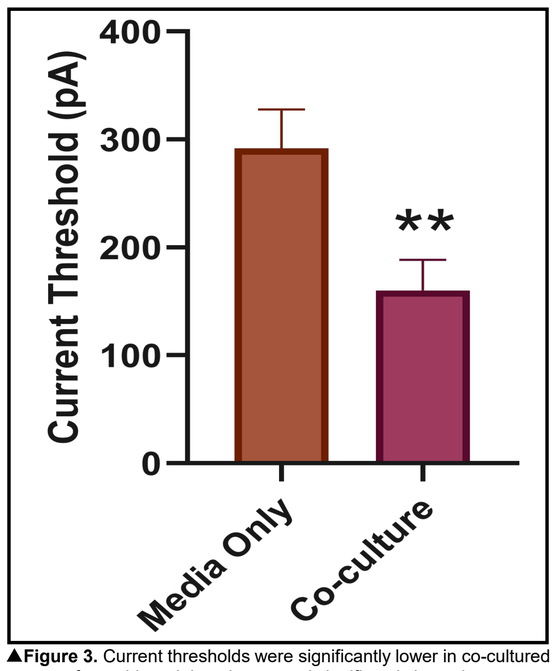
## Results



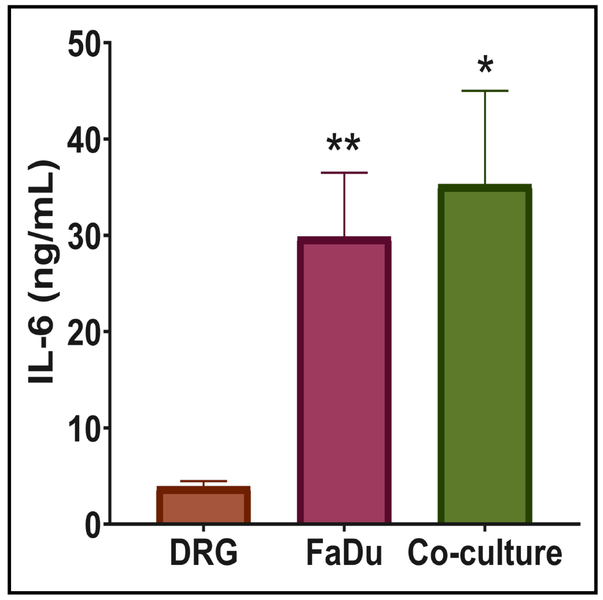

▲**Figure 1.** Co-cultured neurons from older adult male rats demonstrated spontaneous activity (SA) and depolarizing spontaneous fluctuations (DSFs) more frequently than media only control neurons. Increased spontaneous activity and large (>5mV) DSFs indicate neuronal sensitization, which could indicate enhanced nociceptive activity following exposure to Fadu cancer cells in vitro.



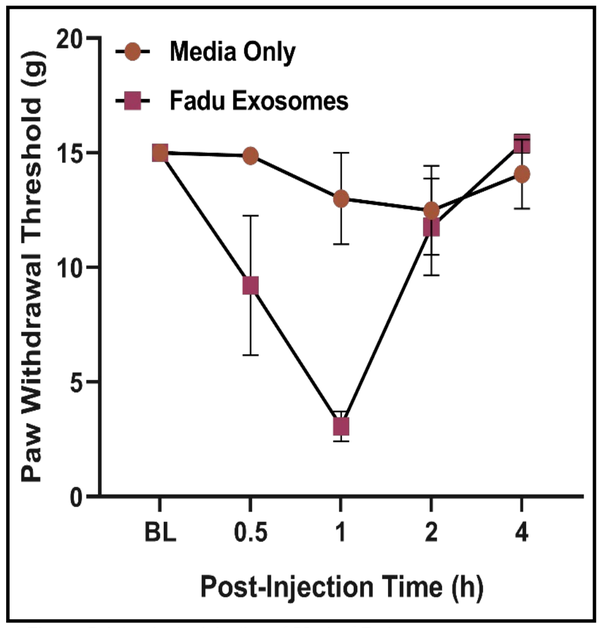

▲**Figure 2.** Intrathetical injection of 30 uL of exosomes isolated from Fadu squamous cell carcinoma induced mechanical allodynia compared to the same volume of phosphate buffered saline (PBS) containing 5% exosome-depleted media.



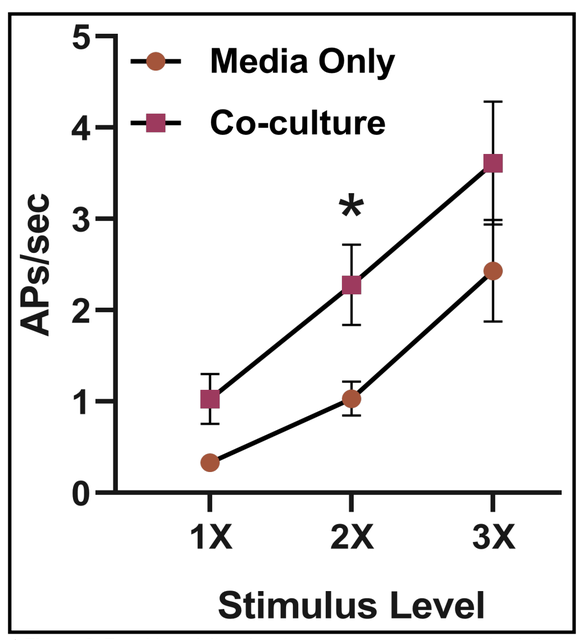

▲**Figure 3.** Current thresholds were significantly lower in co-cultured neurons from older adult male rats and significantly lower in co-cultured neurons with SA compared to those without SA, indicating that exposure to Fadu cancer cells increased sensitivity in vitro.



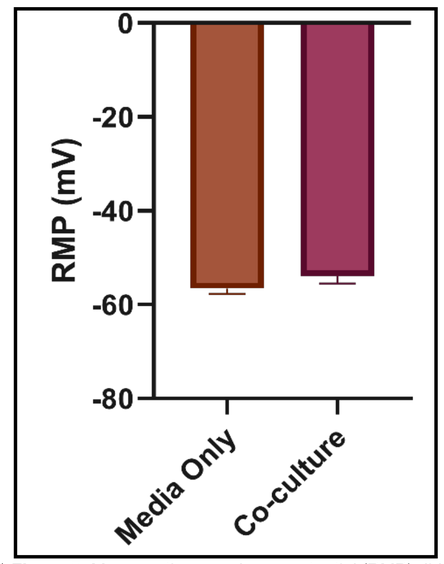

▲**Figure 4.** Chemiluminescence assays show increased expression of the pro-inflammatory cytokine IL-6 when DRG neurons were co-cultured with FaDu cancer cells and when exosomes were released by FaDu cells.




▲**Figure 5.** Intraplantar injection of 50 uL of Fadu exosomes or an equal volume of exosome-depleted media induced mechanical allodynia, indicating that exosomes released from Fadu cancer cells may contribute to enhanced nociceptive activity in vivo.




▲**Figure 6.** For older adult male rats, responses to current stimulation at and above threshold were significantly higher in co-cultured dorsal root ganglia neurons. Post-hoc tests (Bonferroni) showed that this difference was significant at 2X rheobase.




▲**Figure 7.** Mean resting membrane potential (RMP) did not differ between co-cultured and media only neurons in older adult males, but membrane potential was significantly higher in co-cultured neurons with SA compared to those without SA.

## 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

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