Role of tumor-released small extracellular vesicles in cancer pain

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INTRODUCTION

Pain is the most common and earliest symptom of head and neck cancer (HNC) and is reported by about 80% of patients. Despite its prevailing presence in the lives of HNC patients, a treatment providing adequate pain relief is yet to be discovered. Small extracellular vesicles (sEVs) are membrane particles released by cells for intercellular communication. It has been established that sEVs are involved in cancer cell-neuron communication; however, the underlying mechanism of their role in cancer pain isn't fully understood.

HYPOTHESIS

We hypothesized that cancer-derived sEVs communicate with neurons to contribute to cancer pain.

METHODS

To model HNC in mice, oropharyngeal epithelial cells from C57Bl/6 mice were isolated and transformed into cancer cells (mEERL cells). Immunocompetent mice implanted with mEERL cells developed tumors and quickly developed cancer pain, characteristics true to those of human HNC. Control mice received a saline injection. To test sEVs' contribution to cancer pain, genetically modified mEERL cells unable to release sEVs (mEERL Rab27a$^{-/+}$ and Rab27b$^{-/-}$ cells) were injected into mice. These were generated by using CRISPR-cas9 to knock out RAB27a and RAB27b genes involved in sEV release. Finally, SEVs themselves were injected into healthy mice.
Pain sensitivity was assessed.

FIGURES

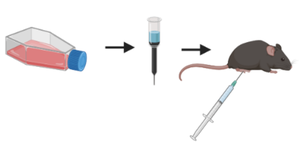

Figure 1: Isolation and injection of cells. Cancer cells were cultured then isolated before being injected into the hind leg of the mouse.

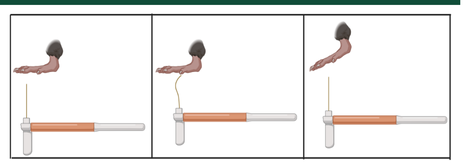

Figure 2: Measuring pain sensitivity by von Frey (vF). A pain threshold was found by obser ving reactions when applying filam ents of gradually increasing or decreasing force to the mouse's hind foot.

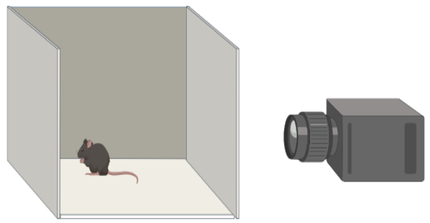

Figure 3: (Left) Measuring spontaneous pain by Mouse Grimace Scale (MGS). The presence of spontaneous pain was assessed by observing facial features including orbital tightening, nose bulge, cheek bulge, and ear position.

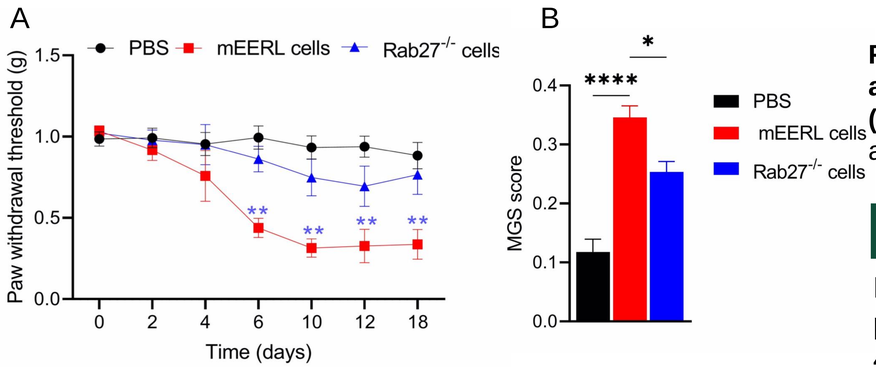

Figure 4: Pain in mice injected with mEERL cells and Rab27b-/- cells. Pain hypersensitivity (A). Pain spontaneity (B).

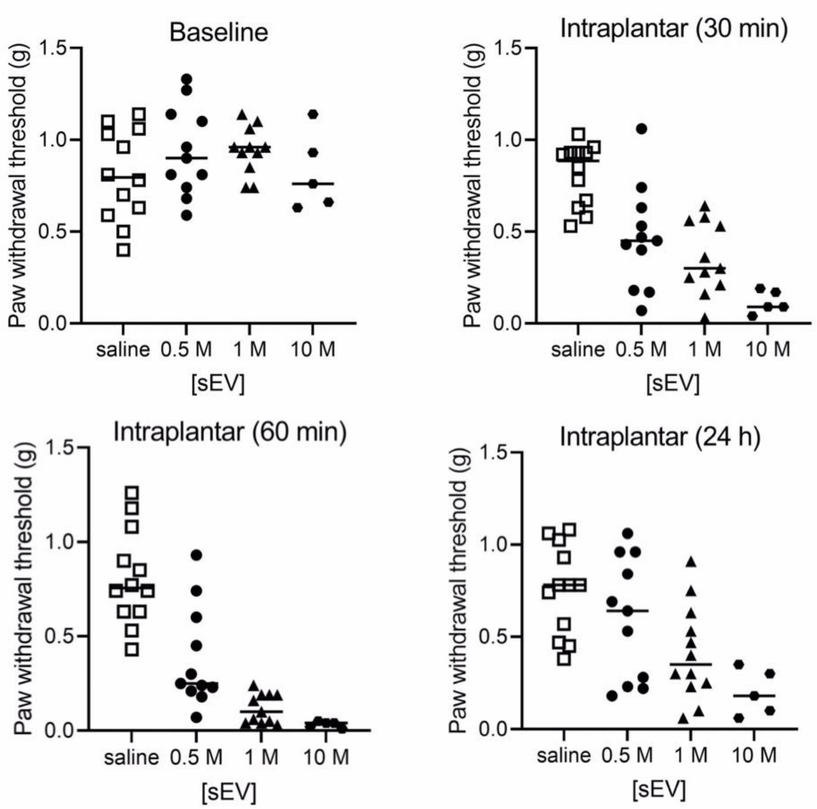

Figure 5: Injection of isolated sEVs induced pain hypersensitivity. von Frey tests were performed at baseline, 30 minutes, 60 minutes, and 24 hours after injection.

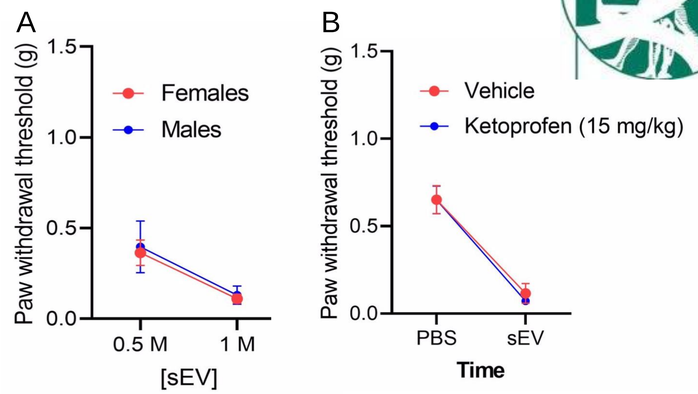

Figure 6: Pain hypersensitivity in response to sEVs is not affected by sexes (A) or anti-inflammatory agent (ketoprofen) (B). Pain sensitivity was assessed 45 minutes after ketoprofen injection.

RESULTS

Mice injected with mEERL cells quickly developed pain hypersensitivity and spontaneous pain (Fig. 4). Mice injected with modified mEERL Rab27b-/- cells, unable to release sEVs, experienced significantly reduced pain compared to mice injected with unmodified mEERL cells.Pain hypersensitivity consistently increased with increasing doses of isolated mEERL sEVs injections (Fig. 5) and is similar in male and female mice (Fig. 6A). The injection of ketoprofen (classical anti-inflammatory drug/pain killer) did not affect the pain hypersensitivity in response to cancer-derived sEV injection (Fig. 6B).

CONCLUSIONS

Our results indicate that isolated sEVs were sufficient to induce pain and blocking sEVs release reduced cancer pain. Ketoprofen is an anti-inflammatory drug. Therefore, its inability to reduce pain hypersensitivity showed that pain did not result from inflammation. Taken together, our data indicate that cancer-derived sEVs are critical mediators of cancer pain and therefore appear as new therapeutic targets for cancer pain.

FUTURE DIRECTIONS

The purpose of cancer cell-neuron communication is not understood and continues to be studied. Additionally, further investigation into the mechanism by which sEVs sensitize neurons is needed. The sEVs used were isolated from cancer cells from male mice; a future direction could be assessing the difference between sEVs isolated from male versus female mice to confirm that sEV response is not affected by sex.