Effects of Chemotherapy on the Taste Stem Cell Microenvironment

Abstract

Why does chemotherapy lead to taste loss?

- Common cytotoxic drugs result in changes in taste function in patients, with consequences for recovery. As taste renewal is mediated by stem cell activity, we hypothesized that chemotherapy may change the stem cell environment, affecting the pattern of taste progenitor cell proliferation.

How can we investigate stem cell activity in the taste organ?

- We investigate how spatial distribution and proliferation status change in response to the chemotherapy drug cyclophosphamide, associated with taste loss in patients.

How and where are taste receptor cells (TRCs) renewed by stem cell activity?

- We discovered a pattern of proliferation in cells along the basement membrane of fungiform papillae (FGPs). We investigate how chemotherapy treatment affects this pattern of proliferation and, in doing so, we hope to elucidate the effects of taste stem cell microenvironment disruption on proliferation status and location. This knowledge may provide a basis for development of treatments for taste loss associated with chemotherapy, COVID-19, and aging.

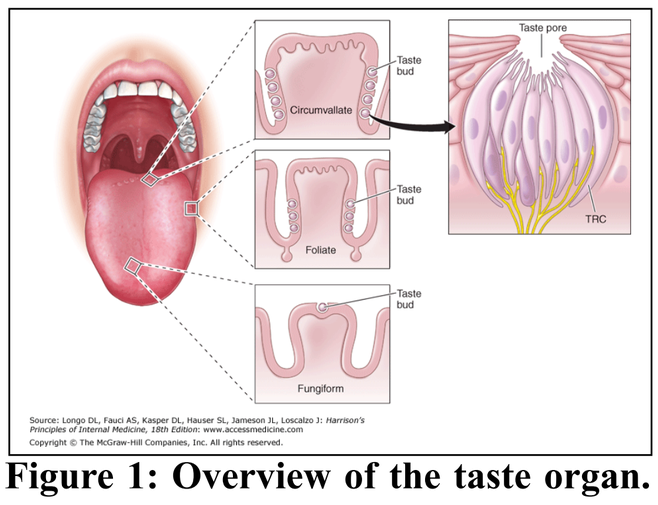

Figure 1: Overview of the taste organ. Taste information is transmitted by taste receptor cells (TRCs) within taste buds, which are housed in fungiform papillae (FGPs), lingual structures located on the tongue.

Methods & Materials

Tongue tissue samples from mice were processed with immunofluorescence staining for cell cycle analysis. Two methods were employed to increase spatial information and statistical accuracy, respectively.

- 1) paraffin sections and 2) whole mount.
- The following markers were used: Ki67 and EdU (cell proliferation), K15 and K17 (candidate stem cell markers), K8 (taste receptor cell [TRC] marker), DAPI (cell nuclei).
- Images were acquired on confocal microscope (LSM800) and analyzed with Fiji (NIH).

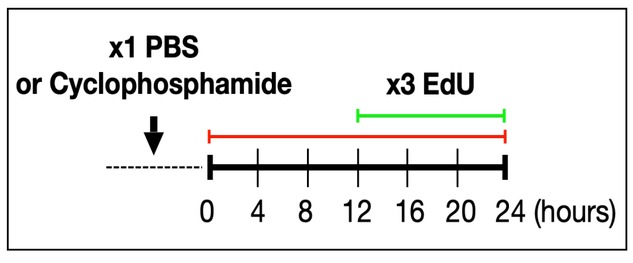

Figure 2: Injection schedule. Following an initial injection of PBS or cyclophosphamide (250 mg/kg), mice were injected thrice with 50 mg/kg body weight EdU (5-ethynyl-2'-deoxyuridine) every 4 hours prior to tissue collection. (Original image.)

Proliferation Status Analysis

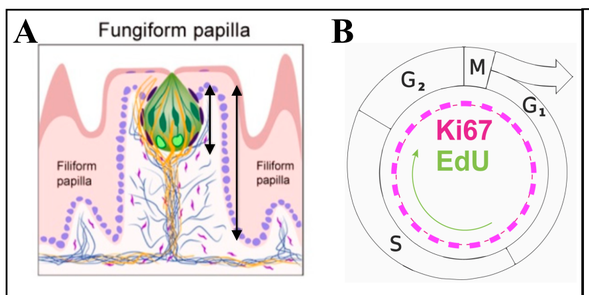

Figure 3: Proliferation status analysis. A) Long and short walls of fungiform papillae (FGPs) selected for analysis. (Source: Longo DL et al.) B) Ki67 marks active cycling cells; EdU marks cells in the S phase of the cell cycle. (Original image.)

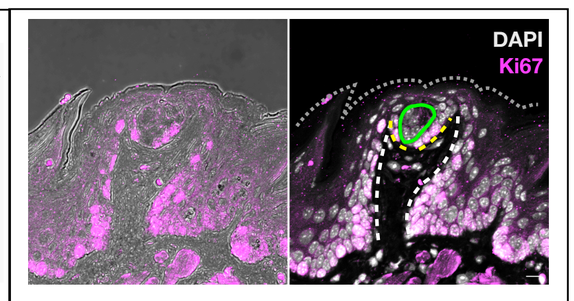

Figure 4: Labeling proliferating cells along FGPs. Location of long wall (white), short wall (yellow), TRCs (green), and tissue (gray); immunostaining with Ki67 (magenta) and DAPI (gray). (Original image. Scale bars, 10 µm.)

Uneven Distribution of Proliferation

A

	Length (micron)
Long wall	91.69 ±4.3
Short wall	33.51 ±0.9

B

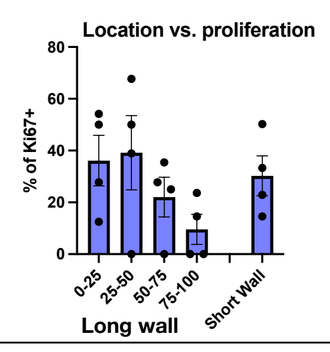

Figure 5: Distribution of cell proliferation along the basement membrane. A) Average length of long and short walls of fungiform papillae (FGPs) (n=22 FGPs from 4 animals). B) Basal region (0-50%) of the long wall and short wall housed the greatest percentages of proliferating cells (n=12 FGPs from 4 animals). (Original image.)

Chemotherapy Reduces Proliferation, Regardless of Location

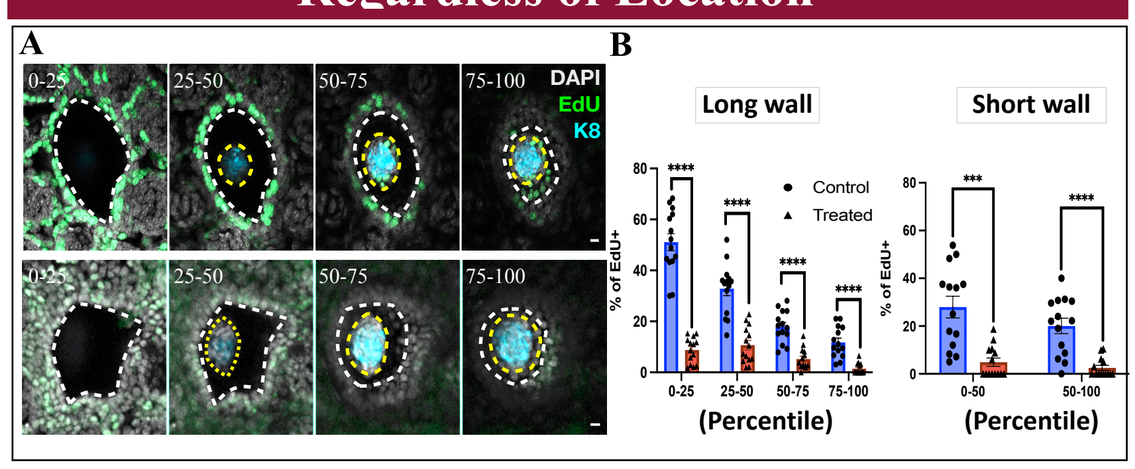

Figure 6: Effects of cyclophosphamide treatment on spatial distribution of proliferating cells. A) Wholemount samples were divided into four regions along the z-axis corresponding to location along the basal membrane. Nuclei adjacent to the basement membrane belonging to the long wall (white dashed line) and the short wall (yellow dashed line) were quantified. B) Chemotherapy treatment resulted in statistically significant changes in proliferation status along both the long and short walls (n=28 FGPs from 2 animals). (Original image.)

Identifying Stem Cell Niches

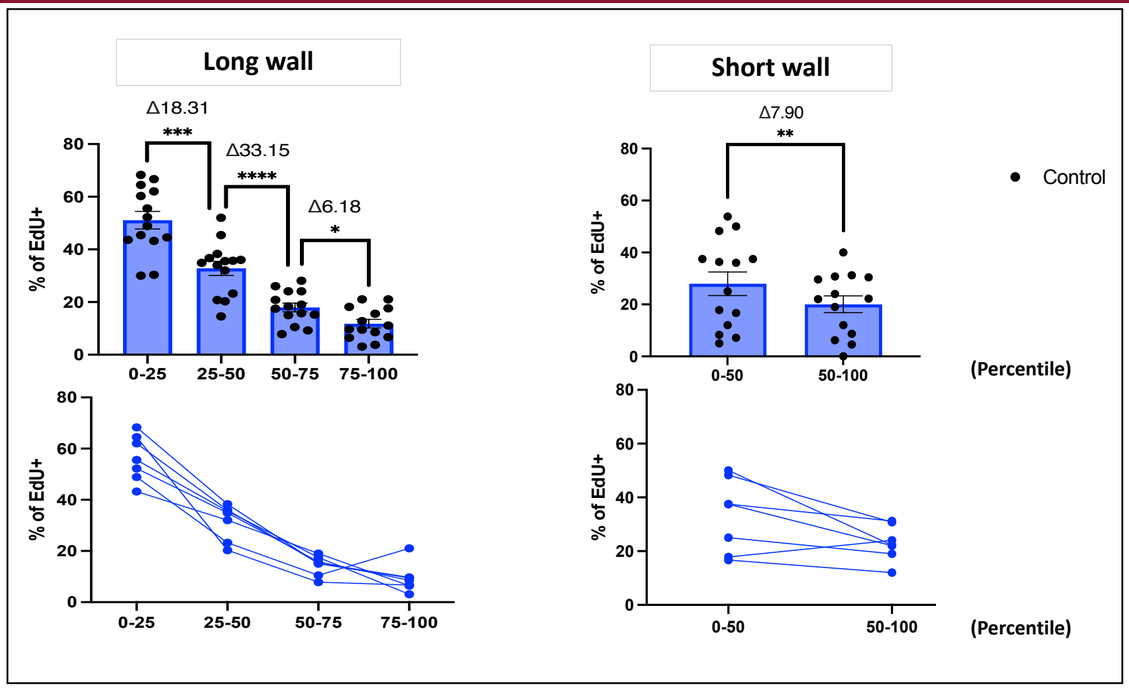

Figure 7: Validation of uneven distribution of proliferation. The wholemount data confirmed the greater percentage of proliferation along the basal region of the long wall and further revealed a similar pattern of activity in the short wall. (Original image.)

Candidate Biomarkers for Stem Cell Niches

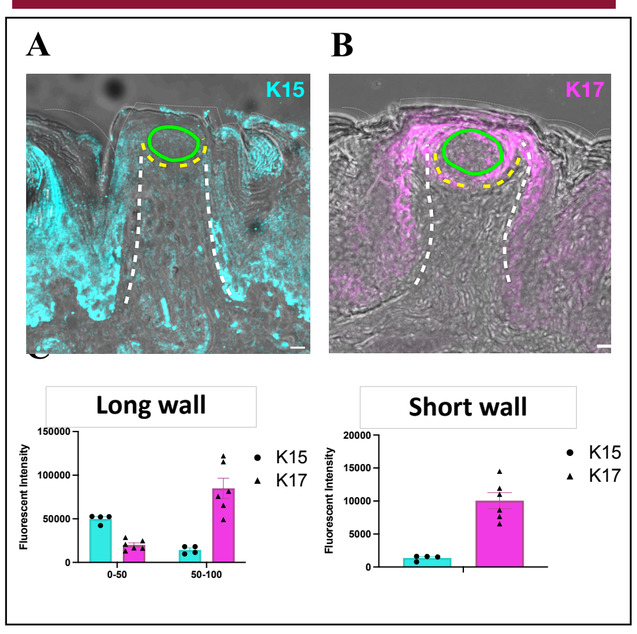

Figure 8: K15 and K17 are candidate markers for identified stem cell niches. A) K15 stains the basal region of fungiform papillae (FGPs). B) K17 stains the apical region of FGPs, complementary to K15. C) Fluorescent intensity of K15 (n=8 FGPs from 2 animals) and K17 (n=10 FGPs from 4 animals) coincide with the newly identified pattern of proliferation.

Conclusions

- Despite an initial uneven distribution of proliferation under untreated conditions, cyclophosphamide reduced proliferation, regardless of cell location.
- We identified distinct markers coinciding with proliferation propensity, suggesting that maintenance of the taste organ is mediated by a heterogeneous population of stem and

Future Directions

- Assess potential location markers under injured conditions
- Perform single-cell analysis to explore additional stem cell and microenvironment markers
- Apply proliferation analysis to samples exposed to COVID-19
- Explore whether location on the tongue may affect proliferation location in mouse models by comparing samples from the intermolar eminence (IME), below the IME, and the tip of the tongue

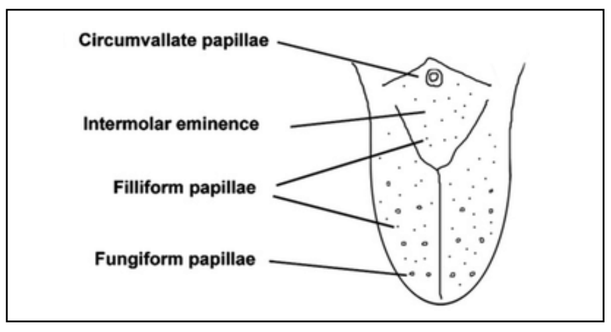

Figure 9: Locations of interest along the mouse tongue. FGPs can be found across three regions of the mouse tongue, with varying degrees of gustatory activity, depending on location. (Source: Kawasaki et al.)