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学位授与の要件	医歯薬学総合研究科機能再生・再建科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Exploring the Role of Ccn3 in Type III Cell of Mice Taste Buds （マウス III 型細胞における Ccn3 の機能の探索）
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学位論文内容の要旨

Taste cells are essential for detecting and processing taste stimuli, with different types playing distinct roles in the perception of taste. These cells can be identified by their unique molecular markers, which enable researchers to study their functions and underlying mechanisms. Using single-cell RNA sequencing (scRNA-Seq) on mouse fungiform papillae, we discovered that Cellular Communication Network Factor 3 (*Ccn3*) was highly expressed in Type III taste cells but not in Type II cells. CCN3, also known as NOV, is a protein involved in cell proliferation, angiogenesis, tumorigenesis, and wound healing. Despite its known roles in other systems, its function in taste cells remained unexplored. Our study aimed to characterize the expression of *Ccn3* in mouse taste buds and investigate its potential role in taste perception and related physiological processes.

To validate the findings from scRNA-Seq, we employed molecular and histological techniques, including reverse transcription polymerase chain reaction (RT-PCR), in situ hybridization, and immunohistochemistry (IHC). These analyses confirmed that *Ccn3* is predominantly expressed in Type III taste cells, both at the transcript and protein levels. This specific expression pattern suggested that *Ccn3* might play a role unique to Type III cells, which are responsible for transmitting sour taste signals and forming synapses with gustatory neurons.

To explore the functional significance of *Ccn3*, we used *Ccn3* knockout (*Ccn3*-KO) mice and conducted a series of experiments comparing them with wild-type (WT) controls. First, we examined the expression of canonical taste cell markers for Type II and Type III cells using quantitative real-time RT-PCR and IHC. Meanwhile, we quantified the numbers of Type II and Type III cells through IHC. No significant differences were observed between *Ccn3*-KO and WT mice, indicating that the loss of *Ccn3* does not affect the differentiation or maintenance of taste cell types.

Next, we investigated whether *Ccn3* influences taste perception. Gustatory nerve recordings were performed to measure neural responses to basic taste modalities (sweet, sour, salty, bitter, and umami). Behavioral tests, including short-term lick assays, were also used to evaluate taste preferences and aversions in *Ccn3*-KO and WT mice. These experiments revealed no significantant differences

in taste responses, suggesting that *Ccn3* is not essential for basic taste function. To gain further insights into the potential roles of *Ccn3*, we conducted bioinformatics analyses based on published scRNA-Seq data. These analyses predicted that *Ccn3* might be involved in tissue regeneration, pain perception, protein secretion, and immune response. Among these functions, the involvement of *Ccn3* in immune processes appeared most plausible based on our experimental results.

In summary, our study indicates that although *Ccn3* is strongly expressed in Type III taste cells, its knockout did not influence the basic taste response, but bioinformatics provided valuable insights into the possible role of *Ccn3* in taste buds and shed light on future research directions.

論文審査結果の要旨

[Introduction] Different taste cells express unique cell-type markers, enabling researchers to distinguish them and study their functional differentiation. Using single- cell RNA-Sequence (scRNA-Seq) of taste cells in mouse fungiform papillae, Cellular Communication Network Factor 3 (Ccn3) was found to be highly expressed in Type III taste cells but not in Type II taste cells. Ccn3 is a protein-coding gene involved in various biological processes, such as cell proliferation, angiogenesis, tumorigenesis, and wound healing. Therefore, this study examined the expression and function of Ccn3 in mouse taste bud cells.

[Methods] scRNA-Seq of fungiform papillae was conducted to demonstrate the specific expression of Ccn3 in taste bud cells. To investigate Ccn3 expression and function, reverse transcription polymerase chain reaction (RT-PCR), in situ hybridization (ISH), immunohistochemistry (IHC), and Quantitative Reverse Transcription PCR (qRT-PCR) were used. Functional studies were conducted using gustatory nerve recordings and short-term lick tests. Bioinformatics analyses were performed to predict potential functions of Ccn3.

[Results] Ccn3 was confirmed to be highly expressed in Type III taste cells. However, Ccn3 knockout (Ccn3-KO) mice showed no significant differences in taste cell marker expression, taste cell regeneration, and taste responses compared to wild-type controls. Bioinformatics analyses suggested potential roles for Ccn3 in tissue regeneration, pain perception, protein secretion, and immune function, with the immune function being the most plausible based on experimental observations.

[Conclusion] This study indicates that although Ccn3 is strongly expressed in Type III taste cells, its knockout did not influence the basic taste response. However, bioinformatics provided valuable insights into the possible role of Ccn3 in taste buds and shed light on future research directions.

This article has been published in the Journal of Neurochemistry. Therefore, the defense committee hereby accepts this article as a doctoral dissertation in Dentistry/Philosophy.