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Confirming the Identity of Tuft Cells in Mouse Submandibular Glands

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Tuft cells (TCs) are bottle-shaped microvilli projecting chemosensory cells and, following their identification 70 years ago,[1,2] have been connected to immune system function in various epithelial tissues, including intestinal and airway epithelia.[3] Until only recently, TCs had not been shown to be present in mouse and human salivary glands (SGs), by transmission electron microscopy detection occurring in only a handful of studies in the 1980s using a rat[4] and no follow-up work has been conducted to verify their presence and biological significance in SGs of additional species in the last 15 years. This gap in the literature led to our study that conclusively verified the presence of TCs across species. Specifically, we used transmission electron microscopy to verify TC morphology and confocal microscopy to identify POU2F3 (POU class 2 homeobox 3), a transcription factor required for the generation of TCs in various epithelia (*eg*, intestinal and airways). Results indicate that TCs are restricted to the striated ducts of submandibular glands (SMGs) in mice, pigs, and humans.[5] Having established TCs' presence in SMGs, the next step is to investigate whether they share features with TCs found in other organs and their biological significance. Therefore, this research letter expands on TCs in SMGs.

We used confocal microscopy to detect the expression of chemosensory, inflammatory, and cholinergic markers known to be co-expressed by TCs in intestine and airways.[3] Our results demonstrate the co-expression of G protein subunit alpha transducing-3/α-

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Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2023.08.010.

Conflicts of Interest:

The authors disclose no conflicts.

Ethical Statement:

The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Reporting Guidelines:

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gustducin (GNAT3, chemosensory marker), interleukin (IL)-25 (inflammatory marker), and choline acetyltransferase (cholinergic marker) in TCs within SMGs from mice (Figure A). Particularly, GNAT3 is a protein involved in TC chemosensory transduction pathway, while IL-25 is a key driver of immune responses in intestine and airways.[3] Furthermore, TCs use choline acetyltransferase to synthesize acetylcholine in intestine, where this neurotransmitter is an essential regulator of fluid secretion. Interestingly, acetylcholine elicits saliva secretion when activating muscarinic receptors on SGs. Still, it is yet unknown if TCs regulate saliva secretion. Together, these findings provide additional evidence confirming TC identity of POU2F3^{+} cells within SMGs and show that these cells share similar features across different organs.

Previous studies suggest that TCs and ionocytes are the same cell type in adults SMGs.[6] In order to clarify this point, we used confocal microscopy for specific cell markers for TCs (ie, POU2F3) and ionocytes (ie, Forkhead Box I1, FOXI1). Our results confirmed that TCs and ionocytes are different cell populations as indicated by the presence of mature TCs (POU2F3^{+}), mature ionocytes (FOXI1^{+}), and a small hybrid TC/ionocyte population (POU2F3^{+}/FOXI1^{+}, Figure B). This provides evidence for branched lineage which proceeds from basal cells (POU2F3^{+}/FOXI1^{+}) to both mature TCs and ionocytes (expressing only POU2F3 or FOXI1, respectively) or transdifferentiation between these 2 cell types. Accordingly, previous studies revealed clues for the immediate relationship between these 2 cell types in respiratory epithelium, as evidenced by the co-expression of POU2F3 and FOXI1 in immature basal cells.[3] Also, it has been observed a reduced expression of FOXI1 in *Pou2f3*$^{-/-}$ mice, showing that ionocytes depend on the presence of TCs via transdifferentiation.[3] Still, in-depth transcriptomic, proteomic, and functional studies consider TCs and ionocytes as distinct cell types when fully differentiated.[7] Together, these findings indicate that TCs and ionocytes are different cell types in SMGs and their specific functions in SG physiology and disease need to be subject of future studies.

Previous studies have demonstrated that TCs activate immune responses in the intestine and airways when exposed to pathogens as indicated by an increase in the number of TCs and secretion of IL-25.[3,8,9] Specifically, IL-25 stimulates innate lymphoid cells to release IL-13, which in turn provokes epithelial remodeling as evidenced by expansion of TCs together with mucus-producing goblet cells, resulting in parasite removal through the exacerbated production of mucus.[3,8,9] In these organs, TCs sense and signal via the taste receptor signaling machinery by activation of GNAT3 followed by calcium efflux that triggers release of signaling molecules such as IL-25, eicosanoids, and acetylcholine from intracellular stores. These molecules then mediate immune cell activation, among other functions.[3,8,9] Interestingly, confocal microscopy analysis for POU2F3 and a ductal marker (cytokeratin 7) results show an increased number of TCs in the striated ducts in SMGs from a mouse model of Sjögren's disease (SD, an autoimmune condition that causes hyposalivation, Figure C).

Interestingly, another study showed that there is an increased number of IL-25^{+} cells in the SMGs of a mouse model of SD and in minor SGs from human patients with SD.[10] Specifically, treatment with IL-25 neutralizing antibody reduced the progression of SD-like features (eg, lymphocytic infiltration) in their mouse model of SD.[10] While the mechanisms for the observed effects are as-yet unknown and the group did not determine the identity of

the IL-25$^+$ cells,[10] we believe that these cells are bona fide TCs. Thus, although speculative, it is reasonable to hypothesize that TCs within SMGs abnormally recruit inflammatory cells resulting in aggregates of lymphocytic cells that are essential for SD pathogenesis. In fact, these events within SMGs would mirror the immune-related events in intestine and airways where TCs promote the expansion of inflammatory cells and the release of inflammatory molecules.[3] Still, further studies will be necessary to confirm or refute this hypothesis.

The main limitation of this article is the lack of mechanistic studies and no evaluation of the possible relation between TCs and SMG function. However, these drawbacks are mitigated by the fact that our new findings prove that TCs within SMGs share common features with TCs from different organs. Also, mature TCs and ionocytes divergent cell identity in SMGs corroborates with prior evidence in different organs[7] and serves as basis to believe that these 2 cell types exert distinct functions in SMGs. Moreover, the increased number of TCs in SD mimics what is observed in different epithelia,[3] which is expected to be related to the recruitment of inflammatory cells. As such, while our previous study was focused only on the detection of TCs across SMGs from different species, the current study demonstrates that TCs express chemosensory, inflammatory and cholinergic proteins and that they are different cell populations from ionocytes. Furthermore, our work links TCs to the pathogenesis of SD by showing that TC number is increased with the progression of the disease, a finding that mirrors the triggering of local immune responses observed in other organs (eg, intestine and airways).[3,8,9] Building on these results, further transcriptomic, proteomic and functional studies will be critical to unveil the biological significance of TCs in SMG structure and function, both in healthy and diseased states, with the ultimate goal of determining the mechanisms by which the observed immunological functions occur therein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Transparency Statement:

Data, analytic methods and study materials will be made available to other researchers from the corresponding author upon reasonable request.

Abbreviations used in this paper:

IL	interleukin
POU2F3	POU class 2 homeobox 3
SD	Sjögren's disease
SG	salivary gland

SMG	submandibular gland
TC	tuft cells

References

1. Rhodin J, et al. Z Zellforsch Mikrosk Anat 1956;44:345–412. [PubMed: 13353477]
2. Jarvi O, et al. Acta Pathol Microbiol Scand Suppl 1956;39:72–73. [PubMed: 13372265]
3. Kotas ME, et al. Annu Rev Pathol 2023;18:311–335. [PubMed: 36351364]
4. Sato A, et al. Anat Rec 1988;220:239–251. [PubMed: 3284416]
5. Tavares Dos Santos H, et al. J Histochem Cytochem 2022;70:659–667. [PubMed: 35993302]
6. Mauduit O, et al. Cell Rep 2022;39:110663. [PubMed: 35417692]
7. Montoro DT, et al. Nature 2018;560:319–324. [PubMed: 30069044]
8. Ualiyeva S, et al. Sci Immunol 2021;6:eabj0474. [PubMed: 34932383]
9. Gerbe F, et al. Nature 2016;529:226–230. [PubMed: 26762460]
10. Guggino G, et al. Arthritis Rheumatol 2018;70:1265–1275. [PubMed: 29569854]

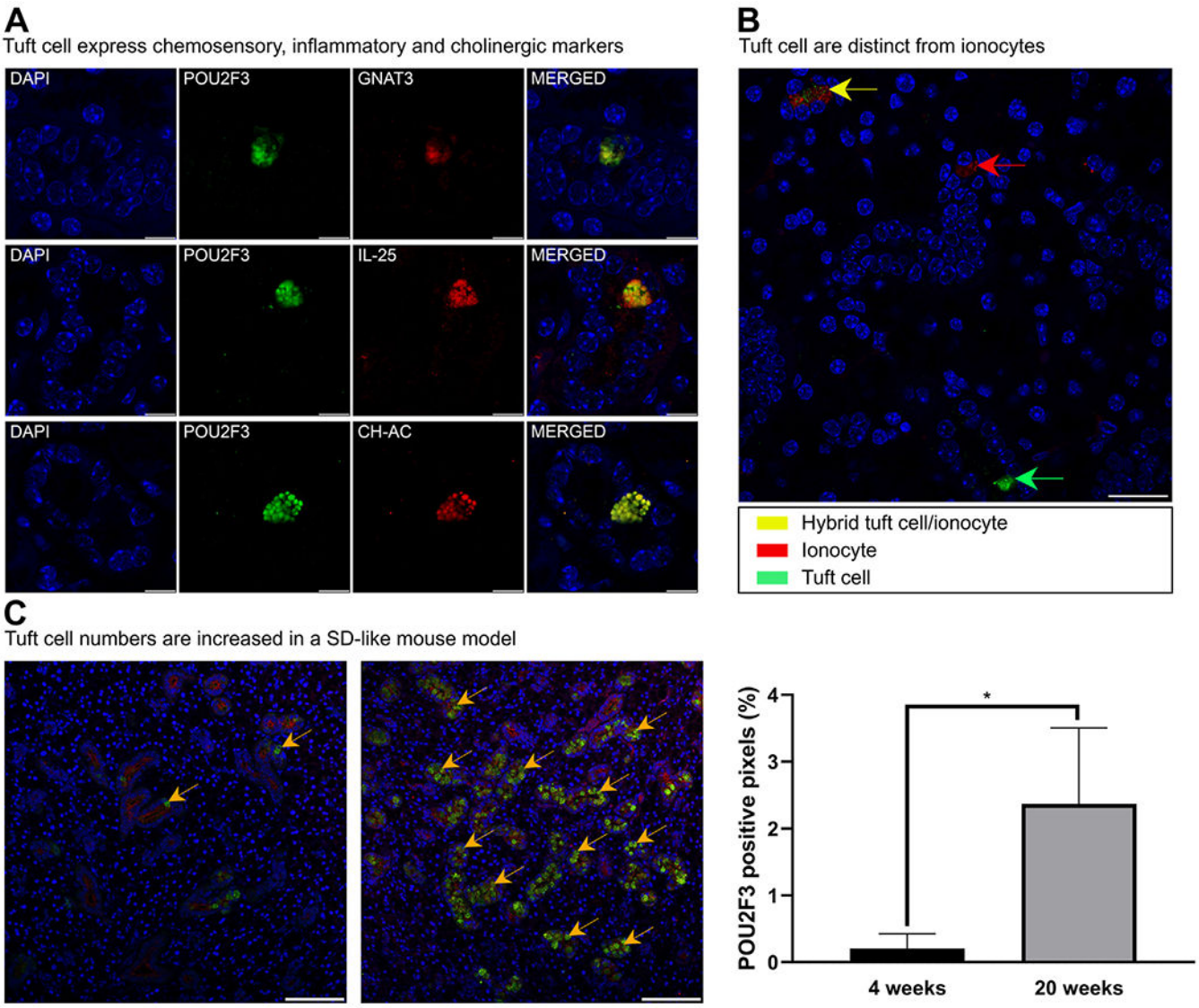

Figure.

Tuft cells (TCs) are a complex population within submandibular glands (SMGs) from mice. (A) TCs express markers related to chemosensory (GNAT3), inflammatory (IL-25) and cholinergic (Ch-Ac) pathways within SMGs from C57BL/6J mice. Scale bars = 10 μm. (B) Three cell populations are detected within SMGs from C57BL/6J mice: mature TCs (POU2F3$^+$, purple arrow), mature ionocytes (FOXI1$^+$, white arrow) and a hybrid TC/ionocyte population (POU2F3$^+$/FOXI1$^+$, yellow arrow). Scale bars = 25 μm. (C) The number of TCs is increased within the SMGs from a mouse model of Sjögren's disease (SD), where TC counts inside cytokeratin 7$^+$ ducts (orange arrows) of NOD/ShiLtJ mice are significantly elevated at disease onset (20 weeks) when compared to pre-disease at 4 weeks (t-test, where *$P < .05$). Scale bars = 100 μm. Images are representative of n = 3 from different animals per analysis.