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Tuft Cells: Context- and Tissue-Specific Programming for a Conserved Cell Lineage

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Abstract

Tuft cells are found in tissues with distinct stem cell compartments, tissue architecture, and luminal exposures but converge on a shared transcriptional program, including expression of taste transduction signaling pathways. Here, we summarize seminal and recent findings on tuft cells, focusing on major categories of function—instigation of type 2 cytokine responses, orchestration of antimicrobial responses, and emerging roles in tissue repair—and describe tuft cell–derived molecules used to affect these functional programs. We review what is known about the development of tuft cells from epithelial progenitors under homeostatic conditions and during disease. Finally, we discuss evidence that immature, or nascent, tuft cells with potential for diverse functions are driven toward dominant effector programs by tissue- or perturbation-specific contextual cues, which may result in heterogeneous mature tuft cell phenotypes both within and between tissues.

INTRODUCTION

Tuft cells are chemosensory epithelial cells dispersed throughout the epithelium in most endoderm-derived organs of mammals (reviewed in 1). Early microscopy in intestine and gallbladder noted their prominent tuft of bundled blunt microvilli, leading to their designation as tuft cells in gastrointestinal tissues (2); original names for tuft cells in other tissues (e.g., microvillus cells, brush cells) also reference this prototypical structure. Modern tools have demonstrated similarity in gene expression in these cells across a range of tissues. This has led the field toward the consensus that all transient receptor potential cation channel subfamily M member 5 $(TRPM5)^+$, interleukin $(IL)\text{-}25^+$, and POU domain, class 2, transcription factor 3 (POU2F3)-dependent epithelial cells expressing genes related to taste signaling and eicosanoid biosynthesis are tuft-like cells, regardless of tissue origin or morphological variations. A variety of other proteins, including choline acetyltransferase (CHAT), doublecortin-like kinase-1 (DCLK1), and advillin, are commonly, though not invariably nor exclusively, expressed in tuft cells (1, 3, 4).

The structure of the eponymous tuft gave the first clue that these cells could act as luminal sensors. This hypothesis was later supported by the finding that tuft cells express key constituents of the canonical taste receptor transduction cascade also found in type II taste cells (5, 6). Sensing of tastants by type II taste cells begins with activation of dimerized G protein coupled receptors (GPCRs) that bind sweet [heterodimers of taste receptor type 1 (T1R) member 2 (T1R2) and T1R3], umami (T1R1/T1R3 heterodimers), and bitter (T2R homodimers) tastants (7). This leads to stimulation of the canonical taste transduction cascade via the G protein subunit α-gustducin (GNAT3), phospholipase Cβ2 (PLCβ2), intracellular calcium mobilization, and activation of the calcium-activated cation channel TRPM5 and culminates in ATP-dependent stimulation of gustatory neurons (7). Similarity between tuft cells and type II taste cells led to the original proposal that tuft cells in the gastrointestinal (GI) or respiratory tracts "taste" luminal contents. Recent work has confirmed that luminal sensing is a prominent function of tuft cells in several mucosal tissues. Downstream of sensory activation, the emerging picture of tuft cells as potent cytokine producers and initiators of adaptive epithelial responses has spurred major interest in understanding their function. Though numerous studies have focused on their promotion of aversive responses, tuft cells, like type II taste cells, likely sense both beneficial (carbohydrate/protein sensing) and harmful (bitter and potentially spoiled or poisonous) tastants and integrate a complex array of luminal signals to engage a spectrum of both positive and negative conditioning.

There have been major advances over the last 10 years in characterization, identification, and functional studies of tuft cells in a variety of tissues, including increasing information on physiologically relevant tuft cell functions in humans. Roles for tuft cells have been uncovered in health and disease: specifically in inflammation, injury, metaplasia, and tumorigenesis. Here, we review major identified tuft cell functions, describe how they arise in distinct tissues in health and disease, and discuss how their dominant function in different tissues or disease states may be environmentally conditioned.

OUTPUTS AND FUNCTIONS OF TUFT CELLS

The majority of tuft cells are found at the luminal surfaces of the alimentary and respiratory tracts. The upper airway and upper alimentary tract are highly related, interdependent, and often dual-purpose structures. The primary function of the conducting airways—which share embryological origins with the inner ear, tonsils, and the thymus—is to transmit inspired air to the gas-exchanging surfaces of the lung while limiting access of particulates, chemicals, or microbes to alveolar spaces. The main role of the alimentary tract is uptake of critical dietary

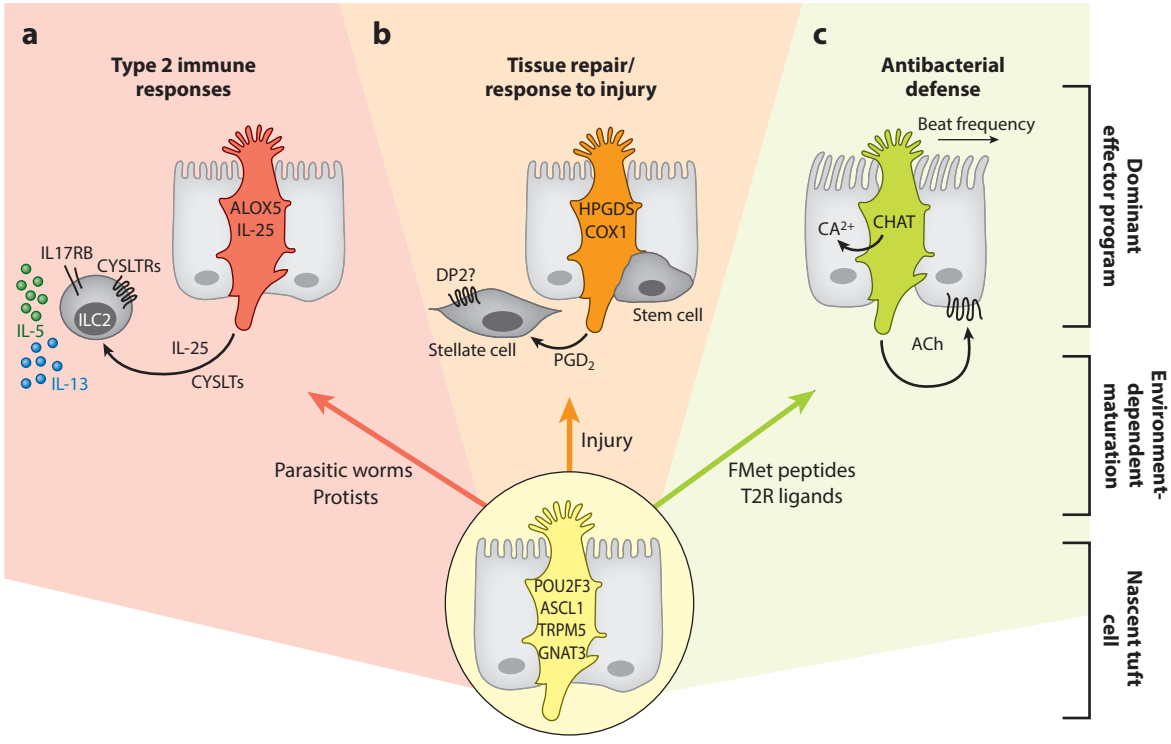

Figure 1

Major functional outputs of tuft cells. Despite the similarity in tuft cell gene expression programs and structure across distinct tissues, tuft cell functions observed in vivo appear tissue and/or context specific. Tuft cell roles can be classified by dominant effector programs and their resulting impact on tissue physiology. (*a*) Major roles for tuft cells in promoting type 2 cytokine responses, specifically from innate/innate-like lymphocytes [group 2 innate lymphoid cells (ILC2s) or type 2 natural killer T cells (NKT2s)] have been found in gut, lung, and thymus. In the gut, tuft cell–mediated production of interleukin (IL)-25 and cysteinyl leukotrienes (CYSLTs) was critical for antihelminth responses, while IL-25 alone promoted adaptive responses to protist colonization downstream of succinate sensing (recently reviewed in 8). (*b*) Roles for tuft cells in tissue regeneration or response to injury have been demonstrated in models of colitis and intestinal stem cell loss (117, 126, 138) and in pancreatitis-induced pancreatic cancer (23). In the injured pancreatic duct, tuft cell prostaglandin D_2 (PGD_2) suppressed inflammatory gene expression in stromal cells and slowed tumorigenesis. (*c*) Antimicrobial defensive function is best characterized in the conducting airways, where bacterial-derived formylated peptides (FMet) or taste receptor type 2 (T2R) ligands induced tuft cell–mediated production of acetylcholine (ACh) and/or lateral calcium release, increased ciliary beat frequency of neighboring epithelial cells, and promoted antimicrobial peptide release (47, 48). There is also evidence for antibacterial roles in the small intestine, via vomeronasal receptor signaling (21), in which tuft cell production of PGD_2 promoted increased mucus release. We suggest that these divergent roles for tuft cells arise as a product of environmental cues (e.g., the presence or absence of activating ligands such as succinate or FMet peptides), promoting environmental-driven maturation of effector functions from nascent tuft cells.

components, while controlling exposure to luminal pathogens. Perhaps unsurprisingly, tuft cells are key epithelial components of both of these dual-purpose organs.

Functional roles described for tuft cells include promoting antimicrobial peptide secretion and mucociliary clearance, instigating type 2 cytokine responses, and facilitating tissue repair. Several tuft cell effector molecules, acting via various responder cells, promote one or more of these responses (**Figure 1**) (also reviewed in 1, 8, 9). While tuft cells have been reported in organs not discussed in depth here (such as the urethra, conjunctiva, and stomach) (1, 10), roles in these organs remain less studied, and their potential functions there may be best surmised from more abundant data in the respiratory tract, alimentary tract, neuroepithelial sensory tissues, and thymus.

Orchestration of Type 2 Immune Responses Through IL-25 and Eicosanoids

The prevailing model of tuft cell function in the small intestine is that they activate tissue-resident innate lymphoid cells (ILC2s) via IL-25 (11, 12), thereby promoting ILC2 proliferation and type 2 cytokine production (IL-5, IL-13), which in turn drives IL4Rα-dependent tuft and goblet cell differentiation from crypt progenitors (reviewed in 9). This circuitry was first demonstrated using rodent models of luminal helminth infection (13–15), where specific tuft cell–activating ligands remain elusive (although T2R signaling may play a role, as demonstrated in *Trichinella* infection) (16). The IL-25-dependent ILC2–tuft cell circuit was also activated by an end product of metabolism from the commensal protist *Tritrichomonas muris*, succinate, which binds the succinate receptor SUCNR1 (GPR91) expressed on small intestinal tuft cells (12, 17, 18). Additional work on protist-mediated tuft cell function suggested that succinate may not be the only ligand responsible for optimal tuft cell/ILC2 antiprotist responses, as this was also impacted by loss of T1R3 expression (19). Notably, neither succinate signaling nor T1R3 was required for antihelminth immunity (17, 19), implicating additional and/or redundant pathways for sensing of these complex pathogens by tuft cells.

Recently, McGinty and colleagues (20) used tuft cell conditional deletion of arachidonate 5-lipoxygenase (ALOX5), the rate-limiting enzyme for production of leukotrienes and a canonical gene in the tuft cell transcriptome, to demonstrate that leukotrienes from small intestinal tuft cells contribute to ILC2 activation and optimal antihelminth immunity. By contrast, tuft cell–derived leukotrienes were not required for succinate-mediated ILC2 activation by protists (16, 19). In addition to leukotrienes, intestinal tuft cells express synthetic machinery for other eicosanoids including prostaglandin D_2 (PGD_2). Sensing of a bacterial metabolite through the vomeronasal receptor Vmn2r26 was identified as a mechanism to stimulate intestinal tuft cell PGD_2 production, which in turn stimulated mucus secretion (21). Consistent with this, tuft cell–deficient organoids produced lower levels of PGD_2 in vitro as compared with wild-type organoids (22). In vivo, loss of the PGD_2 receptor (CRTH2) on hematopoietic cells resulted in impaired ILC2 activation during helminth infection, while loss of CRTH2 on epithelial cells resulted in increased differentiation and reduced proliferation (22). Additional evidence supporting a role for tuft cell–derived PGD_2 in tissue repair is suggested from models of pancreatitis and oncogene-induced pancreatic metaplasia (discussed below) (23). Notably, an alternate effector molecule, acetylcholine (ACh), was recently suggested to promote mucus secretion in mouse gallbladder (24). In this context, PGD_2 levels were actually reduced following tuft cell stimulation and ACh-dependent mucus granule release, a finding that merits further study.

Further study of the ILC2–tuft cell circuit has shown impacts extending beyond acute antihelminth immunity. Consistent with the finding from Schneider et al. (12), multiple groups have now reported that chronic tuft cell hyperplasia with associated ILC2 activation and elevation of type 2 cytokines drive a complex program of small intestinal remodeling, including adaptive gut lengthening (25–28). Kotas et al. (29) recently reported that manipulation of ILC2 function by conditional deletion of the negative regulator CISH (or CIS) led to hyperactive ILC2 cytokine production and concomitant increases in small intestinal tuft cells, at the expense of antibacterial immunity. Similarly, the Diamond lab (30) demonstrated that tuft cell–dependent type 2 cytokine responses in the small intestine during early helminth infection led to increased pathogenesis and dissemination by coinfected flaviviruses. Tuft cell functions in viral infection continue to be actively investigated (see the sidebar titled Tuft Cells in Viral Infection).

Supporting conserved roles for intestinal tuft cells in type 2 immune responses across species, several groups have investigated tuft cells and type 2 cytokines both in nonhuman primates—in which the IL4Rα-driven tuft cell circuit is active (31)—and in human samples, in which tuft cell

TUFT CELLS IN VIRAL INFECTION

In addition to regulating responses to helminths and parasites and impacting antibacterial immunity, tuft cells can also be targets in viral infection. In murine norovirus infection, small intestinal tuft cells are a point of viral entry through viral particle engagement with the surface receptor CD300lf (139, 140). Tuft cells can also be infected with rotavirus (141). In the mouse model of norovirus, tuft cell infection serves to reduce lambda interferon responses, promoting infection and creating a viral reservoir (142). However, tuft cells are not the target cell in human norovirus (143, 144), and the role of tuft cells in human viral infection remains unclear. While tuft cells are not thought to be direct targets for influenza or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as they are with enteric viruses, their expansion following these infections suggests potential roles in antiviral responses, as discussed in the main text.

abundance can be correlated with certain disease states (32–34). For instance, human small intestinal tuft cells were found to express SUCNR1, suggesting that similar circuitry to that described in mice could exist (33). Additional translational work on human intestinal tuft cells would greatly aid efforts to understand whether tuft cells and type 2 cytokines can be manipulated therapeutically to improve intestinal inflammation.

Prior to discovery of their role in the small intestine, the role of tuft cell cysteinyl leukotrienes was investigated in type 2 responses in the airways. *Alternaria alternata* extract challenge led to increased frequency of tracheal tuft cells, an effect that required cysteinyl leukotriene signaling and/or synthesis as well as the ATP receptor P2Y2 on tuft cells, while provision of leukotriene E4 in the airways was sufficient to induce tracheal tuft cell expansion (35, 36). Similar to work in the small intestine (20), tuft cell–derived cysteinyl leukotrienes worked synergistically with exogenous (intranasal) IL-25 to promote type 2 inflammation in lung tissue (37). Additional work is needed to understand the mechanism for IL-25/cysteinyl leukotriene activation of lung type 2 immune responses, since lung ILC2s (unlike intestinal ILC2s) do not express the IL-25 receptor under resting conditions (11). One possibility is that high doses of IL-25 act systemically by inducing activation, egress, and migration of small intestinal ILC2s to the lung (38, 39). Conversely, IL-25 could act primarily in autocrine fashion, as tuft cells themselves express the IL-25 receptor (17). Drawing parallels to their effects on biliary smooth muscle contraction (24), one may surmise that tuft cells could also impact bronchoconstriction—another prominent feature of type 2 disease of the airway—but this remains to be explored. Moreover, the impact of tuft cell–derived prostaglandin E_2 (PGE_2)—recently characterized as a tuft cell effector in the respiratory epithelium (40)—on type 2 responses in the airway has not yet been characterized.

In sum, recent work suggests that tuft cells can secrete multiple substances that may act in synergy to promote type 2 responses. It is not clear whether the same input (for instance, a specific helminth-derived GPCR ligand) drives release of multiple tuft cell effector molecules (e.g., cysteinyl leukotrienes and IL-25), or whether complex organisms such as molds and helminths drive multiple tuft cell outputs because they supply multiple ligands. Recent work in the biliary tree suggests at least one example of a single activating receptor driving multiple tuft cell effector responses (24). It is also unclear whether the deployment of these different tuft cell effectors is temporally or spatially controlled, as discussed further below. Experiments to tease out the potential range of tuft cell functions in a limited tissue niche would be greatly facilitated by identification of additional specific ligand-receptor pairs and the intracellular signaling cascades induced by tuft cell activation.

PROTECTIVE AIRWAY RESPONSES

The nasopharynx and conducting airways are a ready entry portal for inhaled irritants and pathogens. At least three mechanisms can be engaged for airway defense (145). First, bulk movement of air can be controlled through purposeful avoidance, apneic responses, or forcible muscular expulsion by cough or sneeze. Second, noxious material can be entrapped within the mucus layer to be killed or cleared via secreted antimicrobial peptides or ion concentrations and removed via the mucociliary escalator. Mucociliary clearance requires coordinated beating of ciliated cells to move mucus upward, where it can be eliminated via the pharynx through cough or sneeze. Third, the immune system can be engaged for cellular or humoral leukocyte-directed defense. The nose and mouth are not only the anatomic gateway for initiation of aversive airway responses but also functional gatekeepers, informing decisions about bulk ingestion by orchestrating behavioral responses (favoring positive tastes or smells while avoiding those that might indicate toxins or spoilage). While the sensory interface that shapes those preferences is concentrated in the tongue and olfactory system, sensory cues are also relayed from more distal sites to the central nervous system to provide behavioral reinforcement. Current evidence suggests that tuft cells may participate in all of these protective responses.

Mucociliary Clearance and Antimicrobial Responses

Tuft cells, also called brush cells in the conducting airways, have been found to participate in all three of the major routes for airway defense (see the sidebar titled Protective Airway Responses). Bitter taste receptor agonism by acyl-homoserine lactones used for quorum sensing by Gram-negative bacteria stimulate nasal (also known as solitary chemosensory cells) and tracheal tuft cells to release ACh upon nearby peptidergic trigeminal fibers, resulting in both neurogenic mast cell–mediated inflammation and a protective apnea response (41–43). Similarly, administering the T2R agonist cycloheximide to the trachea reduced respiratory rate, potentially via tuft cell release of ACh on adjacent cholinergic neurons (44).

Airway tuft cells have also been suggested to stimulate mucociliary clearance. Depolarizing calcium signals elicited in cultured human nasal tuft cells after T2R activation spread to adjacent epithelial cells, inducing release of antimicrobial peptides (45). This effect was inhibited by concurrent activation of the sweet taste receptor T1R2/3 using sweeteners or bacterial D-amino acids from *Staphylococcus aureus*, leading to enhanced bacterial growth in vitro (45, 46). Similar antimicrobial effects were observed in the gingiva, where tuft cells were implicated in initiation of beta defensin release in the mouth and control of oral microbiota (4). Complementing their antimicrobial functions in the nose and mouth, activation of tracheal tuft cells, including by T2R agonists (47) or formylated peptides (48), accelerated ciliary beat frequency and apical fluid secretion, promoting mucociliary clearance via ACh and/or PGE_2 (40). When taken together, these data support a model whereby bacterial-derived products can trigger tuft cells along the conducting airways to initiate complementary pathways of protection.

It is not clear to what extent tuft cells in the proximal versus distal conducting airways differ in form or function. Taste receptor expression, for example, may differ along a proximal-to-distal gradient (43), but spatial distribution of other receptors has not been examined. Effectors may also differ: While stimulation of mucociliary clearance in the trachea was reported to be ACh dependent, antimicrobial peptide release in the gingiva is likely ACh independent, as these cells lack CHAT expression (4), and in the nasal epithelium, the effect was reported to be mediated by gap junctions (45). While various bacterial products such as acyl-homoserine lactones used for quorum sensing, formylated peptides secreted by invasive bacteria or damaged host cells (49), and bacterial metabolites (21) are reported to act as ligands for T2Rs or other activating GPCRs on tuft

cells, the full array of activating ligands—either bacterial, other microbial, or host-derived—that might inform our view of the roles of tuft cells remains incompletely described. Further, effects of the upper airway microbiome (and, by extension, the causal microbes in aspiration pneumonias) remain little explored.

Immunomodulatory Functions in the Pancreatobiliary Tree

In other gastrointestinal tissues, tuft cells play immunomodulatory and/or reparative roles. ILC2 production of IL-13 was protective against chemical injury in the stomach, and correlated with tuft cell expansion, although the requirement for tuft cells was not tested (50). In the extrahepatic biliary tree, loss of tuft cells led to a microbiome-dependent increase in expression of inflammatory cytokines and chemokines and increased neutrophil recruitment (51), suggesting a role in defense against microbes or response to metabolites. Further supporting this hypothesis, stimulation of tuft cells with the bacterial metabolite propionate resulted in ACh-dependent mucus release and cysteinyl leukotriene-dependent smooth muscle contractions in mouse gallbladder tissue explants (24). These data suggest a sentinel role for biliary tuft cells in response to microbial constituents or by-products, similar to that described in the urethra, gingiva, trachea, and nasal epithelium (4, 48, 52, 53). Finally, while tuft cells are not normally found in the pancreas, they are observed in the pancreatic duct during injury, where they produce PGD_2 that augments myeloid suppressor cells, suppresses fibroblasts, and ultimately limits fibrotic and metaplastic transformation (discussed below) (23, 54).

Immune Education in the Thymus

Specialized thymic epithelial cells (TECs) in the cortex and medulla are critical for development and education of both innate and adaptive lymphocytes, promoting self-tolerance by vetting developing self-reactive T cells for deletion or maturation as T regulatory cells (55). Tuft cells are present in normal thymic medullary epithelium (56, 57) and reside in cornified epithelial structures near Hassall's corpuscles in humans (58). Work in mice suggests thymic tuft cells predominantly regulate type 2 immune responses in the thymus through production of IL-25, although they have also been proposed to present antigen to T cell receptor (TCR^+) cells using major histocompatibility complex class II (MHCII) (58, 59). Detailed analysis of tuft cell–deficient thymic immune cells by Miller et al. (58) revealed that loss of tuft cells led to depletion of IL-4-producing natural killer type 2 cells (NKT2) and IL-4-dependent virtual-memory $CD8^+$ T cells; an additional role for IL-25^+ tuft cells in enforcing immune tolerance to IL-25 was also suggested. While a dominant role for IL-25 is suggested by the findings of aberrant thymic immune environments in both tuft cell–deficient and IL-25-deficient mice and expression of the IL-25 receptor on thymic NKT2 cells (59), roles for other tuft cell effector molecules cannot be discounted. Systemic impacts on immunity caused by the abnormal thymic environment in tuft cell–deficient mice have yet to be described. A further relationship between thymic tuft cells and T cell selection is suggested by data indicating that aberrant thymic antigen presentation itself impacts tuft cell frequency, as described below (60).

Roles in Sensory Neuroepithelia

Tuft cells are found in close proximity to neurons in several tissues including the olfactory epithelium (OE) and vomeronasal organ (VNO). On the basis of similar morphology, gene expression (6), and dependence on the transcription factor POU2F3 (61), type II taste cells in the taste bud [another neuroepithelial tissue with direct interface with the central nervous system (CNS)] may also be considered tuft cells. In addition to their role in distinguishing essential macronutrients

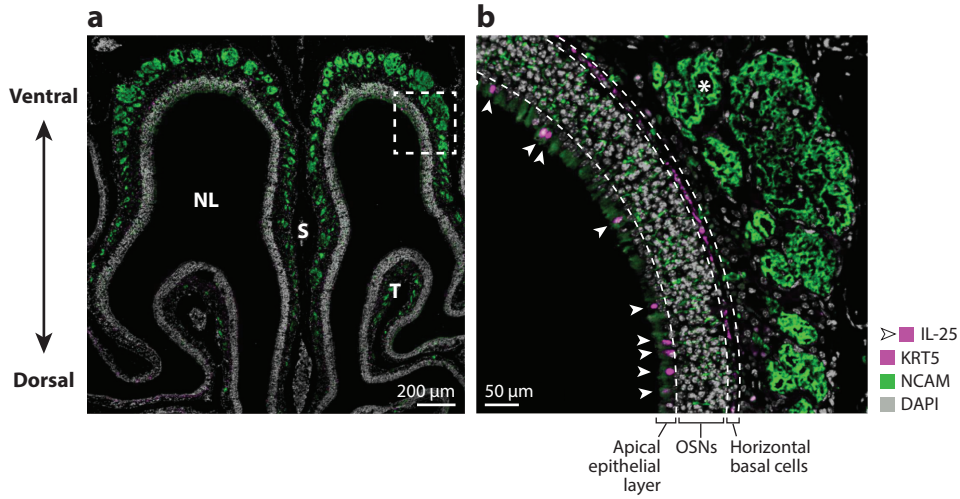

Figure 2

Interleukin (IL)-25$^+$ tuft cells in the olfactory epithelium (OE). Neuroepithelial tissues have critical roles in chemosensation that direct attractive and aversive behaviors. Tuft cells in the OE, taste bud, and vomeronasal organ are in direct contact with presynaptic neurons. (*a*) Confocal image of transverse section from immersion-fixed and decalcified mouse nasal cavity, posterior [nasal lumen (NL), turbinate (T), septum (S)]. (*b*) Inset from panel *a*. OE tuft cells, also known as microvillus cells, are IL-25$^+$ (*magenta*) cells in the apical epithelial layer (marked by white arrowheads), above and in direct contact with olfactory sensory neurons (OSNs). OSNs are observed as a pseudostratified array of nuclei (DAPI stain, *gray*) outlined by neural cell adhesion molecule 1 (NCAM, *green*) processes/cytoplasm, denoted between white dashed lines. A thin layer of keratin 5 (KRT5, *magenta*)-stained horizontal basal cells are also delineated by a white dashed line. An NCAM$^+$ nerve fiber is denoted with a white asterisk.

from toxic ingestions, type II taste cells have also been proposed to have dedicated immunologic function, directing defense against oral microbes via TNF-α (in GNAT3$^+$T1R3$^+$ taste cells) (62) and IL-10 (in GNAT3$^+$T1R3$^-$ taste cells) (63). While type II taste cells in mouse are IL-25 positive (1), no role for IL-25 or eicosanoids in taste chemosensation or in buccal defense has yet been described.

Tuft cells could similarly play roles in both immunity and CNS sensory input in the OE and VNO. OE is found in the posterior nasopharynx directly adjacent to the respiratory epithelium. It serves the dedicated purpose of smell, which also critically contributes to all of the gustatory and emotional phenomena associated with taste. To accomplish this task, neurons of the OE, regenerated throughout life from basal cells, express a vast array of dedicated olfactory receptors and synapse directly on ganglia within the CNS. Tuft cells in OE (also called microvillus cells) reside on the apical layer of the OE (**Figure 2**), in immediate contact with olfactory presynaptic neurons. While OE tuft cells are also IL-25 positive (**Figure 2**), dedicated immune functions for olfactory tuft cells remain unexplored, as in the taste bud.

Unlike in taste buds, tuft cells of the OE do not appear to be dedicated sensors for specific subsets of chemical cues, and mice lacking tuft cells have normal olfactory form and function (64, 65). After olfactory damage, however, tuft cell–deficient mice demonstrated subtle deficits in olfactory-guided behaviors (64), which may point to a role in supporting regeneration of OE. While understudied, such a role would be critically important, because of both the intrinsic importance of olfaction and the potential applications to neuroregeneration in other tissues. Tuft cells in the VNO were proposed to play an additional role in neuronal protection by limiting access

TUFT–NEURONAL INTERFACE

Tuft cell expression of some neuronal genes, their potential to be generated by neuroepithelial basal cells, and their direct interface with neurons in the tongue and olfactory epithelium provoke speculation that tuft cells could serve a perineuronal function and communicate directly with the nervous system in other tissues as well. Indeed, tuft cells have been found to exist in close proximity to nerves in both the airway (41–43, 66) and intestine (34). However, aside from functional evidence supporting interaction with trigeminal nerves in the upper airway (discussed above; see 41–43), direct communication between extraoral tuft cells and neurons remains underexplored. A potential role for tuft–neuronal communication in the bowel is suggested by the report of increased intestinal tuft density in patients with diarrhea-predominant irritable bowel syndrome (34), but this role remains to be further explored. Furthermore, it is interesting to consider the possibility that variation in tuft cell structure between tissues could relate to tuft–neuronal communication. For instance, the direct proximity of olfactory tuft cells to neurons may facilitate their comparatively smaller size and lack of axon-like processes (146), whereas tuft cells in the adjacent respiratory epithelium could require extended cytoplasmic processes to reach beyond the basement membrane to nerves below.

of intranasal compounds to the VNO when bitter tastants were present, an effect thought to be mediated by direct interaction with peptidergic trigeminal fibers (66) and reminiscent of defensive airway protective functions described above. The understudied relationship between tuft cells and neurons is explored in the sidebar titled Tuft–Neuronal Interface.

ORIGINS

The epithelial niches in which tuft cells arise have highly distinct cellular compositions, stem cell compartments, functions, and luminal exposures (**Figure 3**), yet tuft cells across tissues are remarkably similar. This phenomenon provokes questions about the critical requirements and cues that enable tuft cell differentiation from such distinct tissue progenitors. Here, we discuss what is known about tuft cell provenance and physiologic regulators of tuft cell frequency and differentiation under normal and disease states.

Development and Physiologic Regulation of Tuft Cells

Tuft cells are found in diverse epithelial tissues (reviewed in 1), each of which has distinct stem-like progenitors. Here, we summarize major and recent findings on the cellular origins and subsequent differentiation of tuft cells in several well-studied tissues.

Intestine. Development of tuft cells has been best studied in the intestine, where both normal and pathologic conditions have been scrutinized in vivo and in vitro. In both the small intestine and colon, tuft cells are derived from LGR5$^+$ crypt stem cells (67), but their subsequent trajectory differs between the tissues. While their development in the colon is dependent on atonal homolog 1 (ATOH1) (68), several studies have shown that small intestinal tuft cells arise from both ATOH1$^+$ precursors (67, 69, 70) and via an ATOH1-independent pathway (71). ATOH1 conditional deletion in the adult mouse leads to a loss of colonic tuft cells but an increase in small intestinal tuft cells (71). Disagreements regarding the requirement for ATOH1 in tuft cell differentiation (reviewed in 1) were clarified by the discovery that tuft cells can develop independently of ATOH1 via a SOX4-dependent pathway (72), which is responsive to microbial cues including succinate (33). Other work indicated a requirement for cell division control 42 (CDC42), a Rho subfamily small GTPase, and DEAD box-containing RNA binding protein DDX5, which may

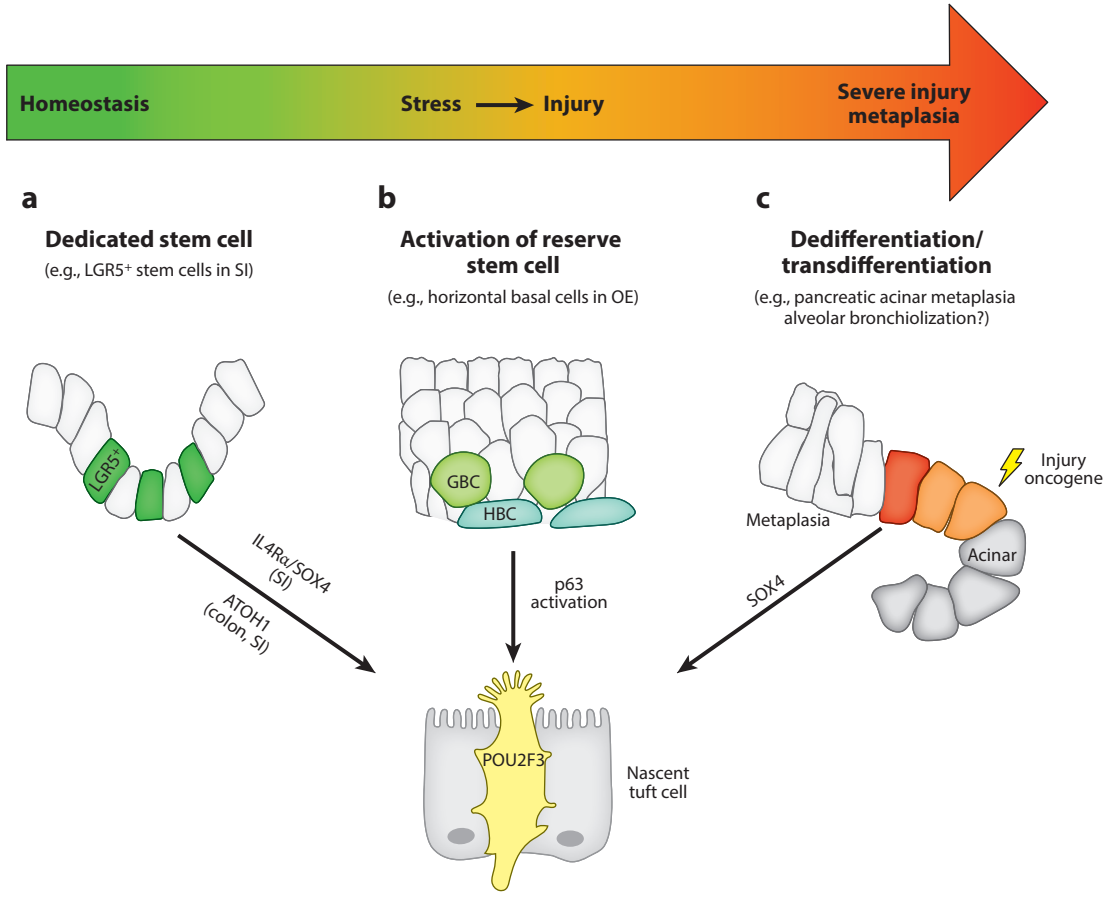

Figure 3

Tuft cell differentiation at homeostasis and in injury. (*a*) During homeostasis, most tissue tuft cells arise from dedicated local stem cells. For example, tuft cells arise from LGR5$^+$ stem cells in both the mouse colon and small intestine (SI). In the SI, this process can proceed in ATOH1-dependent or -independent fashion, with both pathways operating under homeostatic conditions. ATOH1-independent tuft cell differentiation may be driven by type 2 cytokine signaling in a SOX4-dependent fashion. Analogous to the LGR5$^+$ cells in the intestine, tuft cells in the conducting airways can be traced to KRT5$^+$ basal cells, while those in the olfactory epithelium (OE) can be traced to globose basal cells (GBCs). Local epithelial progenitors remain to be identified in some tuft cell–containing tissues (e.g., the extrahepatic biliary tree). (*b*) After injury of tuft cell–containing tissues, reserve stem cell populations can be mobilized to repopulate tuft cells. Such is the case in methimazole-induced ablation of the OE, where otherwise quiescent horizontal basal cells (HBCs) are activated to renew all OE cells including tuft cells (83–85). (*c*) Injury to tissues where tuft cells are typically absent can also promote de novo emergence of tuft cells. Recent work in injury- and oncogene-induced mouse models of pancreatic ductal adenocarcinoma (94, 95) indicates that under severe injury, fully differentiated acinar cells dedifferentiate or transdifferentiate into tuft cells, passing through a mucinous intermediate. Whether this could be the process driving the emergence of tuft cells in severe lung injury has not been examined. In all cases, the fully differentiated tuft cells are remarkably similar in gene expression and structure.

promote CDC42 levels, in enabling tuft cell specification (73, 74). When the pathway was impaired, *Pou2f3* transcript was lost despite maintenance of both ATOH1$^+$ and SOX4$^+$ crypt cells. An additional route of tuft cell differentiation via PROX1, classically required for differentiation of enteroendocrine cells, was uncovered in the context of long-term muscarinic blockade or epithelial deletion of CHRM3, an intestinal epithelial acetylcholine receptor (75). Characterization of this progenitor and support for a close relationship between enteroendocrine and tuft lineages is further described below.

The relative importance of different tuft cell differentiation pathways may depend on immune cues. The SOX4-dependent pathway may predominate under conditions of abundant type 2 cytokines, which activate progenitors via IL4Rα. Subsequently, bone morphogenic protein (BMP) pathway activation by IL-13 serves as a brake on unchecked tuft cell expansion by suppressing further SOX4 induction (76). In contrast, IL-17 signaling may promote ATOH1-dependent tuft cell differentiation. Recent work showed that IL-17RA (which heterodimerizes with IL-17RC to form the functional receptor for IL-17A/F) on $LGR5^+$ intestinal stem cells promoted differentiation of $ATOH1^+$ precursors and ATOH1-dependent secretory cells, including tuft cells (77). Tuft cells were reduced but not eliminated in the absence of IL-17RA expression on either $LGR5^+$ cells or by inducible deletion using villin 1-cre, although interpretation of these results is complicated by the finding that intestinal tuft cells themselves express both LGR5 and IL-17RA (17). Cumulatively, these data suggest a model whereby the major arms of the immune system might stimulate distinct progenitors to converge on specification of the tuft cell lineage.

Airways. Similar to the intestine, the conducting airway epithelia house multipotent progenitors, referred to as basal cells, which give rise to all of the terminally differentiated cells in the pseudostratified epithelium. Indeed, tracheal tuft cells can be traced from keratin-5 $(KRT5)^+$ basal cells using lineage tracing and single-cell sequencing in mice (78) and bioinformatic modeling in humans (79, 80). Suprabasal cells were recently identified as respiratory epithelial intermediates between basal and differentiated cells (81, 82) (analogous to the transient amplifying zone of the intestine), but it is not yet known whether tuft cells arise from this intermediate state. Tracheal tuft cells arise prenatally and expand significantly postweaning (52). Loss of Toll-like receptor signaling reduced their numbers (52), suggesting that microbial products may be among the physiologic cues driving airway tuft cell development.

Despite transcriptional similarity between olfactory, nasal, and tracheal tuft cells (36, 52), olfactory tuft cells arise from distinct stem cell populations that also hold potential to give rise to olfactory neurons. All of the cells of the OE, including tuft cells, arise from one of two olfactory epithelial stem cells: horizontal basal cells, which represent a reserve population that is minimally active in the uninjured state, and globose basal cells, which are responsible for regeneration under most physiologic conditions (83–85). The physiologic cues that inform tuft cell differentiation and determine the tissue set point for olfactory tuft cell density under homeostatic conditions are unknown.

Thymus. In the thymus, tuft cells (a subtype of medullary TECs, or mTECs) likely arise from transiently amplifying mTEC lineage progenitors (86), which derive from self-renewing precursors with both mTEC or cortical TEC potential (87). They appear along with other terminal, post–autoimmune regulator (AIRE) mTECs late in organogenesis, shortly before birth (88), and exhibit both AIRE-independent and AIRE-dependent pathways for development (58). The developmental progression of thymic tuft cells may require the AIRE-binding partner HIPK2, suggesting a role in antigen presentation and self-tolerance (57, 58). Moreover, MHCII-dependent interaction with T cells appears to be a critical facet of thymic tuft cell development and maturation, as mice deficient in this presentation pathway have reduced numbers of thymic tuft cells, while highly self-reactive thymocytes promoted expression of prototypical tuft cell transcripts (60). Lymphotoxin B, a known regulator of mTECs, is critical for development of thymic tuft cells, which express the highest level of the receptor LTBR and fail to develop in the absence of thymic epithelial LTBR; a role for SOX4 is also apparent (59, 89).

Biliary tree. In the extrahepatic biliary tree, the abundant tuft cell compartment has limited turnover in the adult but turns over rapidly in neonatal mice (51). Inducible deletion of tuft cells

resulted in slow recovery of tuft cells, suggesting a local progenitor. In the gallbladder and bile ducts, tuft cell numbers are negatively regulated by bile acids, with reduced tuft cell frequency observed in both dietary and genetic bile acid manipulation; this is further modulated by the presence or absence of the microbiota, which plays an important role in bile acid metabolism (51). Consistent with previous work and the distinct fetal origins of the two tissues (90), no tuft cells were observed in the intrahepatic biliary epithelium, even following cholestatic injury. No studies have yet addressed the relationship between progenitor cells and tuft cells in this tissue.

Tuft Cells Arising Under Injury and Inflammation

Whereas tuft cells are present in many tissues under normal physiologic conditions, they can also arise under conditions of severe injury or during oncogenesis in tissues where they are not normally observed (**Figure 3**). Tuft cells in injury may also take on regenerative roles not otherwise observed.

Inflammation. Many of the same immune cues used under homeostatic conditions can also drive tuft cell expansion under pathologic conditions, such as during the type 2 immune response to intestinal helminth infection (13–15). Tuft cell expansion can be massive during infection with parasites such as the rat-adapted helminth *Nippostrongylus brasiliensis* and facilitate rapid expulsion of the inciting worms, but the mouse-adapted helminth *Heligmosomoides polygyrus* induces a comparatively blunted tuft cell expansion, which may facilitate the parasite's long-term residence in the mouse intestine (91). Such differences may stem from differing degrees of SOX4 activation by IL-13 or from negative feedback through the BMP pathway (76).

The finding that tuft cells during intestinal helminth infection are critical to the activation of innate type 2 responses (including ILC2s) through IL-25 and leukotrienes prompted examination of whether allergic airway disease such as chronic rhinosinusitis with nasal polyps and asthma could also be driven by tuft cells. Indeed, tuft cells were reported to be the major source of IL-25 in the nasal epithelium of polyp patients (92) and were increased in frequency in polyps as compared with nearby healthy tissue as measured by flow cytometry (93). Single-cell sequencing confirmed the increase in tuft cells in nasal polyps, where polyp tuft cells increased eicosanoid synthetic machinery and elaborated PGE_2, which in turn stimulated airway epithelial secretion via CFTR and imparted an associated transcriptional signature (40). Similar expansion of tuft cells was suggested in the lower airways of humans with allergic asthma and during allergic airway inflammation in mice (35–37, 40). How tuft cell–derived factors contribute to allergic airway pathology remains incompletely studied.

Injury. In pancreatitis and oncogene-induced murine models of pancreatic ductal adenocarcinoma (PDAC), DelGiorno et al. (23, 94) demonstrated that tuft cells could transiently arise via transdifferentiation from mature acinar cells and that tuft cell–deficient mice had faster tumor progression. RNA velocity and trajectory analysis further revealed that acinar cells progress through a $TFF2^+MUC6^+$ intermediary progenitor in a SOX4-dependent manner to generate tuft cells (95). In mice, these HPGDS-expressing tuft cells produced PGD_2, which limited development of protumorigenic $ACTA2^+$ fibroblasts associated with worse disease in both mouse and human PDAC in the injured duct (23). Moreover, deletion of GNAT3 in a mouse model of PDAC led to increased presence of myeloid-derived suppressor cells and faster progression to metastasis (54), although this was intriguingly linked to an increased frequency of (perhaps nonfunctional) tuft cells. The presence of mature tuft cells was also observed in human pancreatitis (23, 96), confirming previous work that identified a tuft cell signature in pancreatic metaplasia (97) and suggesting that these mechanisms in mice may be extrapolated to human disease. The appearance of tuft cells in

injury- and oncogene-induced pancreatic metaplasia may offer clues for the normal development of tuft cells from the neighboring biliary epithelium, as pancreatic metaplasia has many hallmarks of biliary epithelium (98). Notably, tuft cells are absent in the pancreatic tumors themselves, both in mice and humans, either because no acinar cells remain to undergo transdifferentiation or because the injury-induced signals for tuft cell specification from a dedifferentiating cell are lost in established tumors.

Echoing their transient expansion during injury and their role in metaplastic progression, stomach tuft cells expanded in number during inflammatory initiation of tumorigenesis (10, 99, 100) before decreasing in the tumor itself. This condition may be associated with a type 2 circuit similar to that in the helminth-infected intestine, wherein tuft cell–derived IL-25 drives IL-13 and metaplastic remodeling and tumor formation (101). As in the pancreas, PGD_2 plays an antitumorigenic role in gastric cancers, but whether tuft cells are the source of PGD_2 in this context has yet to be examined (102). Tuft cells also appear ectopically in Barrett's esophagus, a metaplastic process characterized by progressive replacement of squamous esophageal epithelium with gastric columnar epithelium that includes tuft cells (103, 104), but any role in disease progression has yet to be elucidated.

Tuft cells are normally absent from the distal airways and alveoli in mice. However, they can be found in the honeycombed nests of $P63^{+}KRT5^{+}$ cells (usually limited to basal cells in the conducting airways) that develop after severe influenza-induced lung injury in mice, independent of IL4Rα signaling (105). Recent data has similarly uncovered the ectopic development of tuft-like cells in the alveolar parenchyma of human patients with severe acute respiratory distress syndrome caused by SARS-CoV-2, in parallel with augmented numbers of tuft cells in the airways (106). As in the pancreas, these data support a model wherein severe injury promotes the dedifferentiation of lineage-committed cells that typically lack tuft cell differentiation potential (acinar cells in the case of the pancreas, perhaps type 2 pneumocytes in the case of the lung) toward precursors that can produce tuft cells. Increased pulmonary edema induced by succinate or denatonium administration (107) and decreased myeloid infiltrates in *Pou2f3*$^{-/-}$ mice (106) following influenza-induced injury may suggest that ectopic tuft cells contribute to pulmonary pathology. However, no difference in alveolar regeneration or honeycombing was observed in *Pou2f3*$^{-/-}$ mice (105, 108) indicating that further investigation is needed to establish a function for ectopic tuft cells in pulmonary pathology or repair. Ectopic intrapulmonary tuft cells persist for at least 50 days after influenza infection (107), but it is unclear whether such cells eventually disappear, as in the pancreas. Curiously, intestinal tuft cells were also reported to expand during the acute phase of pulmonary influenza infection, though the functional implications of this epithelial remodeling remain unclear (109).

Neoplasia. Mouse models of pancreatic and stomach injury, and the conspicuous absence of tuft cells from pancreatic tumors following their transient presence during tumorigenic injury (see the section titled Injury, above), suggest that tuft cells may be protective against development of neoplasia. However, despite their absence in established pancreatic tumors, tuft cells also seem to play a role in metastasis, as *Pou2f3*$^{-/-}$ mice subjected to a model of metastatic pancreatic cancer had altered liver metastasis in association with a shifted inflammatory landscape (110). A significant portion of neoplastic growths in the thymus and lung have recently been found to have tuft-like markers. Tuft cells were found to be present at high numbers in some thymic carcinomas (111), as well as in a subset of thymomas (albeit at lower frequency) (112), and even in benign multilocular thymic cysts (113). Using bulk RNA sequencing, the presence of a tuft cell phenotype was positively associated with both SOX9 expression and M2 macrophage markers and negatively associated with tumor-infiltrating lymphocyte markers (114), linking the tuft cell signature with

negative prognostic indicators. Similarly, following the initial observation that a tuft cell signature could be found in a subset of patients with small cell lung cancers (SCLCs) (115), a portion of lung adenocarcinomas and squamous cell carcinomas (despite variable histologic features) were also discovered to have tuft-like markers (111). While data are limited, one study reported that SCLCs with tuft-like signatures were associated with better patient survival and increased chemotherapeutic response (116), contrasting with data in thymic malignancy. Given the normal restriction of tuft cells to central airways lined by the respiratory epithelium, it would be informative to learn whether all tuft-like tumors are more common in central than peripheral pulmonary locations, as reported in SCLCs (115). However, it is notable that tuft cell markers were not overrepresented in medullary as opposed to other thymic tumors, suggesting that restriction of tuft cells to anatomically or histologically appropriate sites is likely breached, even in benign neoplastic disease. Driver mutations for tuft-like tumors have not yet been identified.

In addition to the work discussed above in injury-induced metaplasia (pancreas and stomach) and human neoplasia (lung and thymus), tuft cell gene and protein signatures have been observed in intestinal tumors in both humans and mouse models (117, 118) as well as in human head and neck cancers (119). The vast majority of these studies to date have focused on expression of DCLK1, which promotes tumor invasiveness, epithelial-to-mesenchymal transition (EMT), and metastasis (118, 120, 121). DCLK1 can drive numerous signaling cascades critical in EMT and is associated with negative clinical outcomes in cancer (122). Although an excellent marker for tuft cells in mice, DCLK1 alone is insufficient to infer tuft cell identity, particularly in humans (1). Highlighting this, DCLK1 expression in pancreatic tumors is associated with poor clinical outcomes following resection (123), but the most current evidence suggests that expression is disconnected from the presence of bona fide tuft cells (95, 124).

A recent translational study used patient-derived colorectal cancer samples for in vitro analysis of the renewing properties of tuft-like cancer cells (125). Using the IL-25 receptor IL-17RB—a robust marker for tuft cells at homeostasis—for lineage tracing, the authors demonstrated self-renewal of POU2F3$^+$ IL-17RB$^+$ cells in vitro and in a xenograft model (125). These conflicting reports on tuft-like cells in cancer pathology highlight the need for further research in which (*a*) tuft cell identity is verified through high-resolution imaging of tuft cell structure or transcriptional profiling, (*b*) transcriptional trajectory analysis or lineage tracing is performed to understand whether a tuft cell is the initiating cancer clone or sustaining the tumor, and (*c*) tuft cell deletion studies (e.g., POU2F3, TRPM5, or IL-25 driven) are performed in vitro and in vivo.

Expansion and/or ectopic growth of tuft cells under conditions of inflammation and injury contrast with evidence that, under homeostatic conditions, tuft cells are rare, solitary cells and exhibit minimal growth or turnover in many adult tissues (78, 117, 126). Possible models by which the otherwise constrained specification of tuft cells could be enabled by inflammatory cues include dedifferentiation of lineage-restricted cells to a precursor with increased potency (95) [consistent with models in other tissues (127)], awakening of potent but otherwise dormant progenitors (e.g., stimulation of olfactory horizontal basal cells) (84), and/or emergence of transient progenitors, such as in influenza-damaged lungs (107) (**Figure 3**). While it remains unclear whether tuft cells that emerge during pathology have consequence or purpose in injured tissue, discovery of the soluble or contact-dependent signals that allow for these processes may critically inform understanding of normal tuft cell differentiation and identify new targets for therapeutic intervention.

Tuft Cell Lineage Relationships

Studies of tuft cell development under both homeostatic and pathologic conditions (particularly bioinformatic predictive models applied to single-cell sequencing) have revealed clues to their relationship with other lineages. For instance, in exploring the role of CHRM3 in small intestinal

tuft cell specification, Middelhoff et al. (75) found that tuft cells can differentiate from a PROX1$^+$ precursor, sharing a common lineage with enteroendocrine cells. Building upon this work, p57 was identified as an additional marker of PROX1$^+$ quiescent progenitors in the +4 position of the small intestinal crypt that give rise to tuft and enteroendocrine lineages during homeostatic conditions and can expand potency to repopulate the entire crypt-villus unit during injury (128). Both tuft and enteroendocrine lineages likely depended on SOX4 (72). Similar to studies in the intestine, experiments in the pancreas traced ectopic tuft cell development to a SOX4$^+$ progenitor that also gave rise to enteroendocrine cells after dedifferentiating from acinar cells (95).

Outside of the gut, in vitro studies of the respiratory epithelium also suggested a relationship between tuft cells and both neuroendocrine cells and ionocytes; there, emergence of tuft-like markers in bulk RNA from human air–liquid interface cultures preceded the expression of neuroendocrine or ionocyte markers, and knockdown of *POU2F3* substantially reduced expression of ionocyte and neuroendocrine markers (129). Consistent with these in vitro findings, sampling of respiratory epithelia in vivo identified a small population of differentiating basal cells with markers of all three rare cell types (81). Trajectory modeling in the OE similarly positioned the tuft cell as a precursor to ionocytes (83), and single-cell sequencing in the thymus has also suggested that thymic tuft cells and ionocytes are closely related, although the nature of their relationship is not yet clear (86). Thus, while definitive experiments using fate mapping are incomplete, bioinformatic tools have provided tantalizing evidence for an immediate lineage relationship between these rare cells (tuft cells, neuroendocrine or enteroendocrine cells, and ionocytes) in multiple epithelial tissues.

TUFT CELL HETEROGENEITY

Single-cell sequencing has revolutionized understanding of cellular transcriptional signatures, revealing unappreciated heterogeneity among cell populations previously thought to be homogeneous. This transcriptional heterogeneity holds true even among rare and specialized cells such as tuft cells. In one of the earliest single-cell atlases of small intestine epithelial cells, Haber et al. (130) observed two main transcriptional programs in tuft cells associated with neuronal and immune transcripts, designating these as Tuft-1 and Tuft-2, respectively. Subsequently, tuft cell subsets (transcriptionally distinct from those in the intestine) were observed by single-cell approaches in other tissues, including in the airways (79).

Studies in small intestine have suggested that spatial and temporal drivers of gene expression may underlie tuft cell transcriptional heterogeneity. Initially, microscopy demonstrated that the proportion of cells positive for GFI1B increased as cells advanced up the villus (71). Subsequently, crypt-villus zonation was described using single-cell transcriptomics and laser capture microscopy (131). The markers used to delineate these different zones along the crypt-villus axis were leveraged in an approach called ClumpSeq, which improves rare cell sampling with droplet-based sequencing of small cell aggregates in lieu of single cells (132). Using that method, Manco et al. (132) reported Tuft-2 (immune) transcripts at the villus tip and Tuft-1 (neuronal) transcriptional signatures toward the bottom of the villus. Since the intestinal epithelium is renewed by proliferation of transiently amplifying progenitors near the crypt base that displace older differentiated daughters up the villus, this may indicate that the Tuft-2 signature seen at the villus tip represents a more mature differentiated tuft cell state. Interestingly, the authors also describe enrichment of immune-related transcripts in villus tip goblet cells, suggesting that exposure to luminal signals drives enhanced expression of genes related to immune function in multiple secretory lineages (**Figure 4**). This tuft cell maturation model was further supported using a reporter for GPR46 to mark mature small intestinal tuft cells, in combination with the more ubiquitous intestinal tuft cell marker TRPM5 (133).

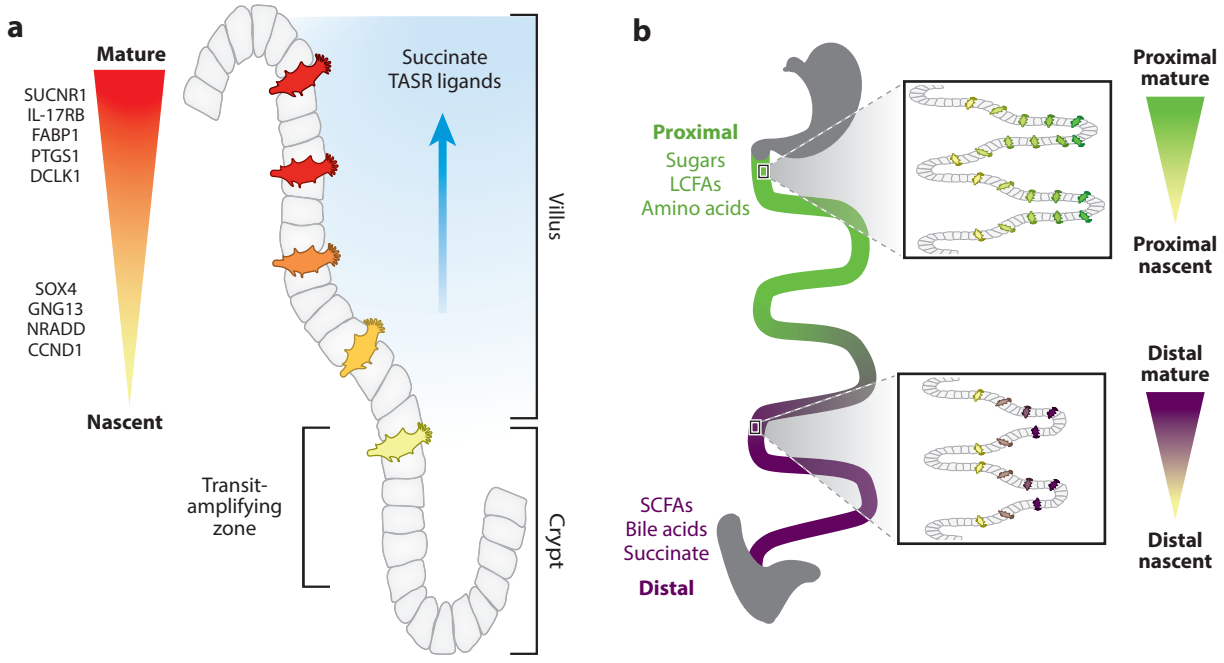

Figure 4

Heterogeneity of tuft cells across space and time. Recent studies using single-cell sequencing and variations on this technique have described heterogeneous gene expression profiles for tuft cells within a single tissue. The biological relevance of this transcriptional heterogeneity remains unknown. We suggest that tuft cell gene expression heterogeneity could represent tuft cell maturation through both space and time, related both to local signaling and environmental cues and to temporal maturation. (*a*) In the small intestine, tuft cell gene expression profiles change along the crypt-villus axis, concordant with cellular age and increasing exposure to luminal contents, including known tuft cell ligands such as succinate. Many transcripts associated with immune function were enriched in tuft cells toward the villus tip (132), while transcripts previously associated with a neuronal phenotype were associated with physical position (pericryptal) or cellular age (newly differentiated): a nascent tuft cell gene signature. (*b*) Tuft cell heterogeneity may also relate more globally to position in the tissue, driven by local environmental cues (e.g., niche-specific stromal cells) and distinct luminal contents. In the small intestine, tuft cells could vary along the proximal-to-distal axis from stomach to cecum/colon, which have highly distinct luminal contents and physiologic functions. Abbreviations: LCFAs, long-chain fatty acids; SCFAs, short-chain fatty acids.

Though best explored in the intestine, the ontogeny of tuft cell heterogeneity has also been examined in the context of the injured mouse pancreas. There, informatic analysis of single-cell sequencing identified multiple tuft cell states along the axis of transdifferentiation from acinar cells during pancreatitis and PDAC (95) and similarly suggested temporal regulation of tuft cell gene expression programs, perhaps analogous to the crypt-villus maturation model in the intestine. This work noted similarity between early stage tuft cells and the neuronal signature identified in Haber et al. (130), while the Tuft-2 signature, including *Il25*, was enriched at later stages. Such data further support a model whereby the tuft cell immune gene program may represent a later stage of tuft cell differentiation and maturation.

In the small intestine, crypt-villus spatial orientation largely corresponds to cellular age following differentiation from LGR5$^+$ crypt cells. Therefore, cellular age or maturation stage proceeds in parallel with increasing exposure to dietary- or microbiome-derived ligands, which may impact tuft cell gene expression programs. While there is no architectural equivalent to the villus-crypt unit in the respiratory epithelium, one may consider how tuft cell heterogeneity could relate to microanatomic locations, such as proliferative hillocks from which basal cells were recently found

to repopulate the injured trachea (79) or polypoid outgrowths in the nose that may expose tuft cells to increased microbial stimulation (40). Likewise, in the extrahepatic biliary tree, where tuft cell heterogeneity was also noted (51), tuft cell transcriptional programs may arise in response to unique luminal exposures in differing anatomic locations, such as in the fundus of the gallbladder, in the cystic duct, or in peribiliary glands in the common bile duct. In the vast majority of cases where tuft cells have been examined at single-cell resolution and heterogeneity has been identified, tuft cells were sampled from macroscopic tissue preparations, with cellular heterogeneity along both the proximal-distal and anterior-posterior axes. Whether tuft cells also vary along these axes is an emerging area of study. The small intestine again serves as a prototypical example where both tissue architecture and luminal exposures (nutrients, microbiota, microbial-derived ligands) change with progression from the proximal duodenum to the terminal ileum (134) (**Figure 4**).

It is important to emphasize that all of the above descriptions of tuft cell heterogeneity have been made possible by application of single-cell sequencing in combination with bioinformatic techniques. The increasing accessibility of spatial transcriptomics will likely improve understanding of how local environments dictate tuft cell transcriptional phenotypes. Development of new tools, such as a temporal fate-mapping approach analogous to that used to track gene expression in differentiating enteroendocrine cells in the small intestine (135), will further improve understanding of tuft cell specification as a function of time. Such tools will also enable correlation between transcriptomes, maturity, variations in cellular structure (for instance, height or complexity of microvilli, secretory vesicles, connections to nearby nerves, or lateral spinules), and functional outputs.

UNRESOLVED QUESTIONS: ROLES FOR NASCENT AND TISSUE-IMPRINTED TUFT CELLS

The remarkable similarity of tuft cell transcriptomes and structures across tissues contrasts with the dramatic differences in their reported functions, which to date are largely segregated by tissue: namely, antimicrobial action and mucociliary clearance effects orchestrated by ACh, PGE_2, and calcium in the airways; IL-25 and eicosanoid-driven activation of type 2 immune responses most prominent in the small intestine; and immunomodulatory responses in the pancreatobiliary system. Since the gene modules for production of all described tuft cell effectors (ACh, IL-25, cysteinyl leukotrienes, prostaglandins) are present in nearly all tissues where tuft cells are found, this leads to the question of how highly distinct, tissue-specific (and, in some cases, injury-specific) stem cell compartments give rise to such transcriptionally similar tuft cells. Once formed with such similar transcriptomes, what cues drive the described tissue-specific functions observed in experimental models? Given the proper signals, do all tuft cells have the capacity for all effector functions?

We posit that the model of progressive maturation of tuft cells within a tissue niche (observed as heterogeneous gene and protein expression) provides clues to these questions. Specifically, we propose that all newly differentiated, or nascent, tuft cells harbor potential for all the effector modules noted in **Figure 1**: type 2 cytokine responses, tissue repair or response to injury, and antimicrobial defense. However, we hypothesize that the specific cues from the tissue niche direct maturing tuft cells toward one or more dominant effector programs. For instance, tissue maturation cues specific to the small intestine may induce expression of SUCNR1 as tuft cells ascend the villus [perhaps defined by spatial guides, such as trophocytes and telocytes (136)]. Acquisition of SUCNR1 expression during villus ascension would be expected to coincide with increasing exposure to luminal succinate, thereby enabling a signaling cascade downstream of SUCNR1 culminating in IL-25 release. Thus, a combination of tissue-derived maturation signals that drive sensory

receptivity and progressive exposure to ligands from the luminal environment could direct a dominant type 2 immune effector program in mature intestinal tuft cells. In further support of such a model, transcriptional signatures of IL-13-dependent epithelial activation were highly correlated with increased tuft cell sensory capability and eicosanoid output in nasal polyposis, suggesting that type 2 inflammatory cues tailor specific tuft cell effector programs (40). The concentration of potential tuft cell ligands would also be expected to vary dramatically from the proximal to distal small intestine, in different areas of the biliary tree, and throughout the anatomy of the airway, perhaps further influencing tuft cell polarization toward their primary effector programs (**Figure 4**). It is unclear whether tuft cell effector programs are continuous, changing in amplitude with increasing or decreasing luminal cues, or are regulated by bistable switches controlling discrete on/off states on the basis of the relative availability of agonists or antagonists.

Also unexplored within the above paradigm is the function (if any) of nascent tuft cells. The immune role of the villus tip Tuft-2 is consistent with the prominent type 2 immune function ascribed to intestinal tuft cells. In contrast, there is no experimental evidence to suggest that Tuft-1 cells play a dedicated neuronal role. Are nascent tuft cells simply immature students of their environment, contributing no outputs but passively absorbing information to shape their eventual profession? Are they present in nascent form to allow for rapid activation when called to duty? Or do the immature and mature tuft cells perform distinct roles, akin to the transition of immune behavior in aging neutrophils (137)? Are nascent tuft cells more poised, for instance, to return to the crypt and stand in for Paneth cells to support the recovering stem cell niche after severe damage (138)? If indeed nascent tuft cells serve a dedicated role, is this a fundamental function shared by nascent tuft cells across all tissues? Once maturation signals are identified that inform the transition from the immature Tuft-1 to the more mature Tuft-2 program as cells ascend the villus, it will be critical to manipulate these signals to probe the respective roles of each tuft cell subset.

If mature tuft cell effector functions are largely dictated by local tissue environments and converge upon a dominant functional output in microanatomic niches, a natural question is whether these functions can change dynamically. Could mature tuft cells shift their function if properly stimulated? And if so, could such stimulations be exploited to suppress tuft cell programs that may contribute to pathology during disease and drive beneficial programs such as to reduce allergic inflammation in favor of regenerative or reparative outputs? This question will prove particularly critical for tissues where epithelial cells exhibit slow turnover and tuft cells may be long-lived (51, 78).

CONCLUSIONS

Cumulative efforts from many labs and new technological innovations have allowed for the unification of cells previously known by many names—tuft cells, brush cells, microvillus cells, solitary chemosensory cells, and type II taste cells—under one shared identity: the tuft cell. These cells share the ability to detect both beneficial and threatening chemical substances, positioning them to act as luminal sentinels capable of integrating diverse environmental cues to reinforce both positive and aversive biological responses. While several major functional programs for tuft cells have been identified, each dominant in specific organ and disease contexts, the full spectrum of tuft cell functions remains to be explored. Meanwhile, descriptions of tuft cell heterogeneity and maturation programs have generated questions about the way contextual environmental cues might shape the effector functions of these unique epithelial cells. Such contexts could relate to differences in polarization and growth factors from stromal or other niche cells, the abundance of activating or suppressive ligands, or immune cues such as IL-13. Addressing such questions will prove critical to understanding the function of these fascinating cells in homeostasis and disease and potentially manipulating those functions toward therapeutic aims.

DISCLOSURE STATEMENT

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