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Tuft cells: Context specific programming for a conserved cell lineage

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Abstract

Tuft cells are found across tissues that have distinct stem cell compartments, varied tissue architecture, and diverse luminal exposures, yet they share a remarkably conserved transcriptional program, including expression of canonical taste transduction signaling pathways. In this review, we summarize seminal and recent findings on tuft cell function, focusing on major categories of tuft cell function which arise downstream of signaling via distinct tuft cells receptors, secretion of several types of tuft cell effector molecules, and activity of context-specific responding cells. We discuss evidence that development of immature "nascent" tuft cells in response to contextual tissue cues drives heterogeneity of tuft cells within and between tissues. We propose that all nascent tuft cells produce core tuft cell effector molecules, while the dominant "effector" phenotype of tuft cells in each tissue and under different perturbations will be shaped by the availability of context-specific cues and heterogeneous responder cells.

Keywords

Tuft cell; type II taste transduction; IL-25; acetylcholine; cysteinyl leukotriene; mucociliary clearance; type 2 immunity

Introduction

Tuft cells are chemosensory epithelial cells dispersed throughout the epithelium in most endoderm-derived organs of mammals (reviewed in (1)). Early microscopy of 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

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other tissues (i.e.: microvillus cells, brush cells) also reference this prototypical structure. Subtle differences in shape between tuft cells in different tissues initially prevented appreciation of tuft cells as a unified cell population. Modern tools, however, have demonstrated remarkable similarity in their gene expression signatures across a range of tissues. This has led the field towards the consensus that all transient receptor potential cation channel subfamily M member 5 (TRPM5)+, interleukin (IL)-25+, 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—first by targeted immunohistochemistry, and later confirmed by transcriptional characterization (5)—that tuft cells express key constituents of the canonical taste receptor transduction cascade (including TRPM5) also found in type II taste cells (6; 7). 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 (8). 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 (8).

The finding that other tissue tuft cells shared many hallmark genes and the transcriptional machinery for GPCR-mediated taste transduction with type II taste cells led to the original proposal that tuft cells in the gastrointestinal (GI) or respiratory tracts “taste” luminal contents. Indeed, recent work has confirmed that luminal sensing is a prominent function of tuft cells in several mucosal tissues. Identifying tissue-specific GPCR expression will be an important step in informing studies on tissue-specific tuft cell function and activation cues, similar to what was found for luminal sensing of succinate by small intestinal tuft cells (9), as discussed below. Downstream of that sensory function, the emerging picture of tuft cells as potent cytokine producers and initiators of adaptive epithelial responses has spurred major interest in understanding the function of tuft cells in diverse tissues and in disease. Based on the spectrum of ligands for type II taste cells, we infer that the ability of tuft cells to sense both beneficial (carbohydrate/protein sensing) and harmful (bitter and potentially spoiled or poisonous) tastants hints at a broad capacity to integrate information about a complex array of luminal signals to engage a spectrum of both positive and negative conditioning.

There have been major advances over the last ten 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. New discoveries in this field have been aided by transcriptomic analysis of tuft cells identified in numerous tissues by high resolution single cell analyses, organoid cultures to model tuft cell functions *in vitro* (10), use of *in vivo* tools (including tuft cell reporter mice (11–13) and specific

Cre drivers for tuft cell manipulation (14; 15)), and recent methods focused on tuft cell isolation and characterization (16; 17). 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. During embryonic development, ectodermal tissue invaginates as olfactory pits and fuses with the endodermal tissue of the developing pharynx, giving rise in the posterior pharynx to pharyngeal pouches lined with endoderm which subsequently generates the inner ears, eustachian tubes and tonsillar lining cells, but also the cortical and medullary epithelia of the thymus. The primitive lung bud bulges more distally from the foregut (18). Once development is complete, the passage from nares through nasopharynx performs most of the work of conducting warmed air to the trachea, while the mouth and oropharynx are responsible for preparing food intake. However, the roles are by no means absolute, with the nasopharynx modulating taste perception, for instance, and the mouth and oropharynx conducting substantial amounts of air. The primary function of the conducting airways is to transmit inspired air to the gas-exchanging surfaces of the lung while clearing it of chemicals, particulates, or microbes that might compromise gas exchange or overall organismal health. The alimentary tract serves an analogous role for nutrients, facilitating and optimizing uptake of critical dietary components while passing non-digestable fibers for excretion, all while maintaining a stable microbiota and subepithelial immune system poised to engage a wide range of pathogens. Perhaps unsurprisingly, tuft cells comprise key epithelial components of both of these dual purpose organs.

Several functional roles for tuft cells have been described to-date, including promoting antimicrobial peptide secretion and mucociliary clearance, instigating of type 2 cytokine responses, and facilitating tissue repair. Several distinct tuft cell effector molecules, acting via distinct responder cells, promote one or more of these responses (Figure 1) (also reviewed in (1; 19; 20)). We note that while the presence of tuft cells has also been reported in other organs not discussed in depth here (such as the urethra, conjunctiva, and stomach) (1; 21) roles there remain understudied, and their function in those organs 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 tuft cells, which seem to be the major source of IL-25, activate tissue resident innate lymphoid cells (ILC2s) by stimulating the IL-25 receptor, IL17RB, which is highly expressed on small intestinal lamina propria ILC2s (22; 23). This IL-25-mediated activation promotes type 2 cytokine production (IL-5, IL-13) and ILC2 proliferation, which in turn drives further tuft and

goblet cell differentiation in response to IL4Rα signaling in crypt epithelial cells (reviewed in (20)). This circuitry was first demonstrated using rodent models of luminal helminth infection (12; 24; 25), where specific tuft cell-activating ligands remain elusive (although Tas2r-signaling may play a role, as demonstrated in *Trichinella* infection) (26). However, this IL-25-dependent ILC2-tuft cell circuit was also engaged in response to succinate, a metabolic byproduct produced by commensals such as the protist *Tritrichomonas muris* during fiber fermentation (9; 23; 27). Small intestinal tuft cells uniquely express the succinate receptor, SUCNR1 (GPR91), which was required for tuft cell – ILC2 responses to *Tritrichomonas* and succinate (9). Additional work on protist-mediated tuft cell function suggested that succinate may not be the only ligand responsible for optimal tuft cell/ILC2 anti-protist responses, as this was also impacted by loss of Tas1r3 expression (28). Notably, neither succinate signaling nor Tas1r3 was required for anti-helminth immunity (9; 28).

Recently, McGinty and colleagues used tuft cell conditional deletion of arachidonate 5-lipoxygenase (ALOX5), the rate-limiting enzyme for production of leukotrienes B4 and C4 and a canonical gene in the tuft cell transcriptome, to demonstrate that leukotrienes from small intestinal tuft cell further aid in activation of ILC2s and optimal anti-helminth immunity (15). Tuft cell-produced leukotrienes were not required for succinate-mediated ILC2 activation by protists (26; 28). In addition to leukotrienes, tuft cells express synthetic machinery for other eicaosanoids, and have ben shown to produce prostaglandin D_2 (PGD_2). In the intestine, sensing of a bacterial metabolite through the vomeronasal receptor Vmn2r26 was identified as a mechanism to stimulate tuft cell PGD_2 production, which in turn stimulated mucus secretion (29). Consistent with this, tuft cell-deficient organoids produced reduced levels of PGD_2 in vitro as compared to wildtype organoids (30). *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 (30). 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) (31).

Further study of the ILC2-tuft cell circuit has shown impacts extending beyond acute anti-helminth immunity. Consistent with the finding from Schneider et al (23), 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 which including adaptive gut lengthening (32–35). Kotas et al 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, which came at the expense of impaired anti-bacterial immunity during acute infection with *Salmonella typhimurium* (36). In a similar vein, work from the Diamond lab demonstrated that tuft cell-dependent type 2 cytokine responses in the small intestine during early helminth infection led to increased pathogenesis and dissemination by co-infected flaviviruses (37). Tuft cell responses in viral infection continue to be actively investigated (**Sidebar 1**).

Supporting conserved roles for intestinal tuft cells across species, several groups have investigated tuft cells and type 2 cytokines in both nonhuman primates—in which the IL4Rα

driven tuft cell circuit is active (38)—and in human samples, in which tuft cell abundance can be correlated with certain disease states (39–41). Although many of these human studies are correlative, data from the Lau lab supports that small intestinal tuft cells in human specifically express SUCNR1, and that loss of tuft cells is correlated with ileitis, implying a protective role for tuft cells in preventing chronic tissue inflammation as demonstrated in a mouse model (40). 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 work demonstrating the role of tuft cell cysteinyl leukotrienes in the small intestine, the role of leukotrienes in airway responses in mice were investigated in response to *Alternaria alternata* extract. There, airway challenge led to increased frequency of tracheal tuft cells, which was lost in the absence of cysteinyl leukotriene signaling and/or synthesis, and provision of leukotriene E4 in the airways was sufficient to induce tracheal tuft cell expansion (42). Further work in this model demonstrated that the ATP receptor P2Y2 on tuft cells was required for the cysteinyl leukotriene response to *Alternaria* (43). However, the mechanism of tracheal tuft cell expansion in this model is unclear, both because tracheal tuft cells were reported to be a source of leukotrienes themselves (43) and because leukotriene receptor-deficient mice were reported to have higher baseline levels of tuft cells. Similar to work in the small intestine (15), tuft cell-derived cysteinyl leukotrienes in the context of exogenous (intranasal) IL-25 synergistically promoted eosinophilia and other indices of type 2 inflammation in the lung (44). How the exogenously provided IL-25 acts remains unclear, since, under homeostatic conditions, lung ILC2s (unlike intestinal ILC2s) do not express the IL-25 receptor (22). One possibility is that high doses of IL-25 act systemically by inducing activation, egress, and migration of small intestinal ILC2s to the lung (45; 46). In that case, the applicability to airway allergy is unclear, as tuft cell-derived IL-25 secretion is likely lower. Conversely, IL-25 could act primarily in autocrine fashion, as suggested by evidence that tuft cells themselves usually express the IL-25 receptor (9).

In all of the above, it is notable that at least in some cases, tuft cells can secrete multiple substances which 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 are able to drive multiple tuft cell outputs because they supply multiple ligands as inputs. Nor is it clear 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 (akin to the identification of succinate), as well as understanding the intracellular signaling cascades induced by different modes of tuft cell activation which lead to production or release of specific effector proteins.

Mucociliary clearance and antimicrobial responses

Tuft cells have been found to participate in all three of the major routes for airway defense (**Sidebar 2**). Stimulation of tuft cells in the conducting airways has been found

to trigger reflexive avoidance behaviors. For instance, bitter taste receptor agonism by acyl-homoserine lactones used for quorum sensing in Gram-negative bacteria were shown to stimulate nasal tuft cells (also known as “solitary chemosensory cells”) to TRPM5-dependent release of ACh upon nearby peptidergic trigeminal fibers, resulting in both neurogenic mast cell-mediated inflammation and a protective apnea response (47–49). Similarly, administering the T2R agonist cycloheximide to the trachea caused a drop in respiratory rate, potentially via tuft cell (aka “brush cell”) release of ACh on adjacent cholinergic neurons (50).

Complementing these bulk clearance and avoidance mechanisms, airway tuft cells have also been suggested to stimulate mucociliary clearance. Depolarizing calcium signals elicited in cultured human nasal tuft cells after TAS2R activation were followed by calcium signaling propagation to surrounding epithelial cells, provoking their release of antimicrobial peptides (51). 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 (51; 52). Similarly, gingival tuft cells were implicated in initiation of beta defensin release in the mouth, and tuft cell-deficient or GNAT3-deficient mice had distinct microbial diversity and accelerated alveolar bone loss as sequelae of the resulting gingivitis) (4). Complementing the provision of antimicrobial functions in the nose and mouth, activation of tracheal tuft cells, either by TAS2R agonists (53) or by formylated peptides sensed through alternate TRPM5-activating receptors (54), can accelerate ciliary beat frequency and promote mucociliary clearance through local release of acetylcholine (ACh). It is not clear to what extent tuft cells in the proximal versus distal conducting airways differ in form or function. Some evidence suggests that taste receptor expression may differ along a proximal-to-distal gradient (49) but spatial distribution of other receptors has not been examined. Outputs 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); in the nasal epithelium this effect was reported to be dependent on gap junctions (51).

When taken together, this body of literature supports a model whereby bacterial-derived products can trigger tuft cells along the conducting airways to initiate complementary pathways to protect the airway. The ways in which the upper airway microbiome (and, by extension, the causal microbes in aspiration pneumonias) may be impacted by the cumulative tuft cell activities in the mouth, nose, pharynx, or trachea has yet to be explored. Moreover, while various bacterial products such as acyl-homoserine lactones used for quorum sensing, formylated peptides secreted by invasive bacteria or damaged host cells (55), and bacterial metabolites (29) are reported to act as ligands for Tas2Rs or other activating GPCRs on tuft cells, the full array of activating ligands—either bacterial, other microbial, or host-derived—which might inform our view of the roles of tuft cells remain incompletely described.

Immunomodulatory functions in the pancreatobiliary tree

In other gastrointestinal tissues, data suggests that 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 (56). 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 (O'Leary et al, in press), suggesting a role in defense against microbes or response to metabolites, such as microbiome-derived secondary bile acids. This data suggests a sentinel role for biliary tuft cells in response to microbial constituents or byproducts, similar to that recently described in the urethra, gingiva, trachea and nasal epithelium, although cholinergic signaling was not examined (4; 54; 57; 58). Finally, while tuft cells are not found in the pancreas during normal circumstances, they were reported to arise during injury, where they produced PGD_2 that augmented myeloid suppressor cells, suppressed fibroblasts, and ultimately limited fibrotic and metaplastic transformation (discussed below) (31; 59).

Immune education in the thymus

Derived from primitive endoderm, specialized thymic epithelial cells 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 (60). Tuft cell are found in normal thymic medullary epithelium (61; 62), and reside in cornified epithelial structures near Hassall's corpuscles in humans (63). Work in mice suggests thymic tuft cells regulate type 2 immune responses within the thymus predominantly through production of IL-25, although direct antigen presentation has also been proposed based on the tissue specific expression of MHCII on thymic tuft cells (63; 64). Detailed analysis of tuft cell deficient thymic immune cells by Miller et al, 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 (63). Systemic impacts on immunity caused by this imbalanced thymic cytokine environment and defective NKT2 cell development 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 (65).

Tuft cell roles in sensory neuroepithelia

Type II taste cells and tuft cells in other organs share more than just the taste transduction signaling cascade. Rather, based on similar morphology, gene expression (6), and in their reliance on the transcription factor POU2F3 (7), type II taste cells in the tastebud can be considered tuft cells. The tongue is a neuroepithelial tissue with direct interface with the central nervous system (conveying taste, as well as a other sensory cues). Since loss of type II taste cells obliterates behavioral responses to sweet, umami, and bitter tastants (7) the neural and behavioral implications of lingual taste cells are profound, though beyond the scope of this review. In short, the ability to integrate chemical cues that report on factors ranging from nutritional composition to risk of harmful ingestion enables these cells to participate in complex positive and negative reinforcing behaviors. In a more narrow framing, lingual tuft cells, like other tuft cells, can also be considered to play a defensive role insofar as taste distinguishes essential macronutrients from toxic ingestions. Type II taste cells have also been proposed to have dedicated immunologic function, perhaps

directing defense against oral microbes via TNFa (in GNAT3+T1R3+ taste cells) (66) and IL-10 (in GNAT3+T1R3- taste cells) (67). While type 2 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 play roles in both immunity and central nervous system (CNS) sensory input in other neuroepithelium, such as the nearby the olfactory epithelium (OE) and vomeronasal organ (VNO). OE is found in the posterior nasopharynx directly adjacent to respiratory epithelium, within the larger structure of the conducting airways. 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 designated “microvillus cells”) reside on the apical layer of the olfactory epithelium (Figure 2), in immediate contact with olfactory presynaptic neurons, although the nature of their communication is not clear. While olfactory 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 apparently have apparently normal olfactory form and function (68; 69). However, following olfactory damage, tuft cell-deficient mice demonstrated subtle deficits in olfactory-guided behaviors (68), which may point to a role in supporting regeneration of OE. While understudied, such a role would be critically important, both because of the intrinsic importance of olfactory sensation, and because of 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 of intranasal compounds to the VNO when bitter tastants were present: an effect thought to be mediated by direct interaction with peptidergic trigeminal fibers (70), and reminiscent of defensive airway protective functions described above. The fascinating and understudied relationship between tuft cells and neurons is explored in **Sidebar 3**.

Origins

While their tissues of origin are largely endoderm-derived, the distinct epithelial niches in which tuft cells arise have highly distinct cellular compositions, stem cell compartments, functions, and luminal exposures (Figure 3). Found most often in simple columnar epithelium, tuft cells are also found in highly structurally distinct tissues comprising, including cuboidal and pseudostratified epithelia. Despite these differences in underlying tissue composition and architecture, remarkably similar tuft cells develop in all tissues. This phenomenon poses a question regarding the 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 conditions and during disease, which may inform hypothesis generation towards this key unanswered question.

Development and physiologic regulation of tuft cells

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 derive from LGR5+ crypt stem cells (71), but their subsequent trajectory differs between the tissues. While tuft cell development in the colon was dependent on atonal homolog 1 (ATOH1) (72), several studies have shown that small intestinal tuft cells can arise both from ATOH1-expressing precursors in the transit amplifying zone (71; 73; 74) and via an ATOH1-independent pathway (75). In contrast to loss of tuft cells in the colon under these conditions, ATOH1 conditional deletion in the adult mouse led to an increase in small intestinal tuft cells (75). Discrepancies in the literature with regard to 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 (76), which is responsive to microbial cues, including succinate (40). ATOH1-independent development of tuft cells may occur predominantly under conditions of abundant type 2 cytokines and active signaling through IL4Rα on progenitor cells. 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 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 *Il17ra* (9). 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.

Recent work on signaling components involved in differentiation of intestinal crypt cells indicated a requirement for cell division control 42 (Cdc42), a Rho subfamily small GTPase, in promoting MAP kinase signaling, in the viability of *in vitro* cultured intestinal organoids and tuft cell specification (78). Supporting this, loss of the DEAD box-containing RNA binding protein DDX5, which may promote CDC42 levels in intestinal epithelial cells, led to a specific reduction in tuft cells (79). Here, use of spatial transcriptomics suggested that the tuft cell lineage, as read out by presence of *Pou2f3* transcript, was lost despite maintenance of both ATOH1+ and SOX4+ crypt cells. This may indicate a later stage commitment to the tuft cell lineage operating in both colon and small intestine that requires CDC42 signaling for activation of the tuft cell program, regardless of which progenitor cell is involved. The specifics on how these two major routes for tuft cell specification and development are regulated remain the subject of active investigation (35; 80). A third 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 (81), as discussed below.

An important consideration in loss-of-function studies, particularly those targeting intestinal stem cell and differentiating progenitors, is that altered gut cytokine responses to normal commensal flora—for example, in tuft cell loss of function models—can lead to

unexpected downstream effects that could impact other intestinal cell types or other tissues directly. Facility-specific flora and dietary metabolites could similarly lead to systemic, gut-dependent effects that complicate data interpretation. Conversely, loss of cell lineages, including tuft cells, in either a global or intestine-specific way, could lead to altered intestinal flora, nutrient acquisition or changes in systemic metabolites which might also impact intestinal stem cell development and physiological outcomes. We strongly advise the use of littermate controls and cohousing for validation of both developmental and functional tuft cell studies in the intestine and elsewhere.

Airways—Similar to the intestine, the resting respiratory nasal and tracheal epithelia house a population of stem cells with multipotent regenerative potential, referred to as basal cells, which can give rise to all of the terminally differentiated cells in the pseudostratified epithelium. Indeed, tuft cells can be traced from Keratin-5 (KRT5)+ basal cells using lineage tracing and single cell analysis of mouse trachea (82) and bioinformatic modeling in humans (83; 84). While respiratory basal cells exhibit heterogeneity that predicts the daughter cells they produce (85), the specific basal cell states that give rise to tuft cells have not yet been identified. Additionally, single cell transcriptomics have helped identify suprabasal cells (86; 87) as respiratory epithelial intermediates between basal and differentiated cells (perhaps analogous to the transient amplifying zone of the intestine), but it is not yet known whether tuft cells arise from this intermediate state. Bioinformatic predictions are limited by the small size of the pool of suspected intermediate tuft cell precursors (86) making it challenging to trace their differentiation. Tracheal tuft cells arise prenatally and expand significantly post-weaning, as demonstrated through use of the Chat eGFP reporter (57). Loss of TLR signaling leads to reduced tracheal tuft cell numbers (57), which suggests that microbial products may be among the physiologic cues that drive airway tuft cell development. Of note, this dependence on TLR signaling was also reported in the urethra (57), where prior work demonstrated a role in limiting ascending bacterial infection (88).

Despite transcriptional similarity between olfactory, nasal, and tracheal tuft cells (43; 57), olfactory tuft cells arise from distinct stem cell populations which also hold potential to give rise to olfactory neurons. All of the cells of the olfactory epithelium, 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 (89–91). The physiologic cues that inform tuft cell differentiation and set the tissue “set point” for olfactory tuft cell density under homeostatic conditions are not yet clear.

Thymus—Tuft cells are abundant among endoderm-derived medullary thymic epithelial cells, or mTECs, which arise from a local self-renewing precursor that has the potential to differentiate into either mTECs or cortical thymic epithelial cells (cTECs) as recently reviewed (92). Thymic tuft cells are predicted to arise from transiently amplifying progenitors that give rise to multiple mTEC lineages (93). Recent mTEC lineage trajectory analysis in the developing thymus in mouse embryos demonstrated that tuft cells appear along with other terminal, post-AIRE mTECs late in organogenesis, shortly before birth (94). Fate mapping studies in adult mice demonstrated that thymic tuft cells exhibit both

autoimmune regulator (AIRE)-independent and AIRE-dependent pathways for development, and are present in *Aire*−/− mice (63). The developmental progression of thymic tuft cells may require the AIRE-binding partner Hipk2, suggesting a role in antigen presentation and self-tolerance (62; 63). Moreover, MHC II-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 (65). More recent work on the heterogeneity of mTECs demonstrated that 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 (64; 95).

Biliary tree—Work in the extrahepatic biliary tree using novel and established fate-mapping tools demonstrated that the abundant tuft cell compartment in this tissue has limited turnover in the adult, but that tuft cells are abundant and turnover rapidly in neonatal mice (O'Leary et al, in press). Consistent with previous work, and the distinct fetal origins of the two tissues (96), no tuft cells were observed among the intrahepatic biliary epithelium, even following cholestatic injury. Inducible deletion of tuft cells resulted in slow recovery of tuft cells, suggesting a local progenitor. However, how tuft cells develop in the gallbladder and bile ducts remains unclear. In the extrahepatic biliary tree, tuft cells 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 (O'Leary et al, in press).

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 usually seen in these post-mitotic cells, as noted below.

Inflammation: Although cells and molecules of the immune system contribute to normal tissue homeostasis, activation of these mediators exists along a spectrum of inflammation, and can reach levels that compromise core tissue functions in support of host defense. Many of the same microbial-induced 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 (12; 24; 25). While 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, the mouse-adapted helminth *Heligmosomoides polygyrus* induces a comparatively blunted tuft cell expansion, which may facilitate the parasite's longterm residence in the mouse intestine (97).

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 has 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 (98), and were increased in frequency in polyps as compared to nearby healthy tissue as measured by flow cytometry (99). Unfortunately, tuft cells were not identified in recent single cell sequencing datasets of type 2 inflamed airway tissue, precluding confirmation of this finding through other means (100; 101). Although there are no accepted models of allergic nasal polyposis in mice, allergic airway disease in the lower conducting airways has been modeled in mice, with one group reporting an increase in tuft cell number during allergic stimulation (discussed above; (42–44)). Similar expansion of tuft cells in the lower airways of humans during allergic asthma has not yet been reported, but the finding that tuft cells are absent from the distal airways in mice (102) would predict that these cells will be rare in bronchioles, even in pathologic settings like asthma. Whether IL-25 or other 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 demonstrated that tuft cells could transiently arise during injury through a process of transdifferentiation from mature acinar cells (31; 103), 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 via a SOX4-dependent manner to generate tuft cells (104). In mice, these HPGDS-expressing tuft cells produced PGD_2, which limited development of pro-tumorigenic ACTA2+ fibroblasts associated with worse disease in both mouse and human PDAC in the injured duct (31). Moreover, deletion of GNAT3 in a mouse model of PDAC led to increased presence of myeloid-derived suppressor cells and faster progression to metastasis (59), although this was intriguingly linked to an increased frequency of (perhaps nonfunctional) tuft cells. The presence of a mature tuft cell lineage was also observed in human pancreatitis (31; 105), confirming previous work which identified a tuft cell-signature in pancreatic metaplasia (106), 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 (107), including the presence of tuft cells. 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 de-differentiating cell are lost in established tumors.

Echoing their transient expansion during injury and role in metaplastic progression, stomach tuft cells expanded in number during inflammatory initiation of tumorigenesis (21; 108; 109) before decreasing in the tumor itself. PGD_2 also plays an anti-tumorigenic role in gastric cancers, although whether tuft cells are the source of PGD_2 in this context has yet to be examined (110). Like their appearance during pancreatic injury, 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 (111; 112). However, the role of tuft cells in progression of Barrett's metaplasia has yet to be elucidated, and whether the appearance of tuft cells in injury

and metaplasia in tissues where they are not normally present is always accompanied by replacement with an epithelium in which tuft cells are normally found is unknown.

Tuft cells are normally absent from the distal airways and alveoli in mice. However, tuft cells can be found in the honeycombed nests of abnormal KRT5+ cells that develop after severe influenza-induced lung injury in mice, independent of IL4Rα signaling (113). These nests form after massive expansion of a rare subpopulation of progenitors that adopt expression of KRT5 and P63, which is normally limited to basal cells in the central airways; influenza-induced ectopic intrapulmonary tuft cells can be lineage traced to KRT5+P63+ progenitors, just as they are in the normal central airways. Recent data has similarly uncovered the ectopic development of tuft-like cells in the alveolar parenchyma of human patients with severe COVID-19, in parallel with augmented numbers of tuft cells in the airways (114). As in the pancreas, these data support a model wherein severe injury promotes the de-differentiation 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 (115), and decreased myeloid infiltrates in *Pou2f3*$^{-/-}$ mice (114) following influenza-induced injury may suggest that ectopic tuft cells contribute to pulmonary pathology. However, no difference in severity of KRT5+ honeycombing in was observed in *Pou2f3*$^{-/-}$ mice (113), suggesting that further investigation is needed to definitively establish a function of ectopic tuft cells in pulmonary pathology or repair. Ectopic intrapulmonary tuft cells persist for at least 50 days after influenza infection (115), but it is unclear whether such cells eventually disappear, as in the pancreas.

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 “injury,” above) suggest that tuft cells may be protective against development of neoplasia. Interestingly, 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 (116). A substantial 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 (117); additionally, although initially thought to be present only in malignant disease, tuft-like cells were subsequently detected in a subset of thymomas (albeit at lower frequency) (118), and even in benign multilocular thymic cysts (119). 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 (120). These data specifically link the presence of a tuft cell signature with negative prognostic indicators, suggesting that tuft or tuft-like cells may themselves contribute to tumor progression. Similarly, following the initial observation that a tuft cell signature could be found in a subset of patients with small cell lung cancers (SCLC) (102), a portion of lung adenocarcinomas and squamous cell carcinomas (despite very different histologic features) were also discovered to have tuft-like markers (117). Unlike in the thymus, the presence of the tuft cell signature has

little association with clinical outcomes in the limited population thus far studied, and no functional effects have been ascribed to these abnormal tuft or tuft-like cells. Given the normal restriction of tuft cells to central airways lined by 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 (102). However, it is notable that tuft cell markers were not overrepresented in medullary as opposed to other thymic tumors, suggesting that restriction to 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 (14; 121) as well as in human head and neck cancers (122). 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 (121; 123; 124). DCLK1 can drive numerous signaling cascades critical in EMT, and is associated with negative clinical outcomes in cancer (125). Although an excellent marker for tuft cells, particularly in mice, DCLK1 alone is insufficient to infer tuft cell identity (1). Highlighting this, DCLK1 expression in pancreatic tumors is associated with poor clinical outcomes following resection (126), but the most current evidence suggests this expression is disconnected from the presence of bona fide tuft cells (104; 127).

In neoplasia specifically, the appearance of a tuft cell gene signature in numerous tumor settings drove early investigation into whether tuft cells could themselves be a reserve stem cell activated in injury, as demonstrated for PROX1-dependent enteroendocrine cells (123), or a cancer stem cell promoting tumorigenesis. The activation of stem cell potential from tuft cells cell remains unclear, as conflicting studies utilized DCLK1-based approaches for tuft cell manipulation in mouse (14; 128), which is complicated by expression of this gene in non-tuft cells as noted above. More recently, a translational study used patient-derived colorectal cancer samples for *in vitro* analysis of the renewing properties of tuft-like cancer cells (129). Using the IL-25 receptor, IL-17RB—a robust marker for tuft cells at homeostasis—for lineage-tracing, the authors demonstrate self-renewal of POU2F3+ IL-17RB+ cells *in vitro* and in a xenograft model (129). The increasing availability of more specific and faithful tools and markers for *in vivo* study of tuft cells in mouse models and continued advances in organoid culture and *in vitro* genetic manipulation of patient derived cells, will no doubt be useful in future work in this area. In sum, the role for tuft cells in tumorigenesis and metastasis remains unclear, particularly given the emerging roles for tuft cells in tissue defense and immune regulation. The conflicting reports on tuft-like cells in cancer pathology highlights the need for further research in which tuft cell identity is verified through high resolution imaging of tuft cell structure or transcriptional profiling; transcriptional trajectory analysis or lineage-tracing is performed to understand whether a tuft cell is the initiating cancer clone or sustaining the tumor; and tuft cell deletion studies are done using tuft cell specific manipulations (e.g.: POU2F3, TRPM5 or IL-25 driven) *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 (14; 82; 128). Possible models by which the otherwise constrained specification of tuft cells could be enabled by inflammatory cues include de-differentiation of lineage-restricted cells to a precursor with increased potency (104) (consistent with models in other tissues (130)), awakening of potent but otherwise dormant progenitors (ex: stimulation of olfactory horizontal basal cells) (90), and/or emergence of transient progenitors such as in influenza-damaged lungs (115) (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 differentation 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 found that tuft cells can differentiate from a PROX1+ precursor, sharing a common lineage with enteroendocrine cells (81). Both cell types likely depended on SOX4 (76). Further insights may emerge by mapping similarity to reported “early tuft” cells subset in heterogenous populations which are more similar to cells in the transient amplifying zone (131). 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 de-differentiating from acinar cells (104). 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 ionocytes markers, and knockdown of *POU2F3* substantially reduced expression of ionocyte and neuroendocrine markers (132). 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 (86). Trajectory modeling in the olfactory epithelium similarly positioned the tuft cell as a precursor to ionocytes (89) 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 (93). 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 previously unappreciated heterogeneity among cell populations previously thought to be homogenous. This transcriptional heterogeneity holds true even among rare and specialized cells like tuft cells. In one of the earliest single-cell atlases

of small intestine epithelial cells, Haber et al observed two main transcriptional programs in tuft cells, dubbing these “Tuft-1” and “Tuft-2”, associated with neuronal and immune transcripts, respectively (133). This observation confirmed previous reports that both types of effector programs were present in intestinal tuft cells when examined in bulk (5). Subsequently, tuft cell subsets were observed by single cell approaches in other tissues, including in the airways (83). Notably, the cluster-specific signatures of tuft cell subsets in small intestine and airway tissues are largely non overlapping, and upstream regulators of these distinct gene expression programs have not been identified.

Further work in small intestine has suggested that spatial and temporal drivers of gene expression may underlie tuft cell transcriptional heterogeneity. Intially, microscopy demonstrated that the proportion of cells positive for Gfi1b (an early marker for intestinal tuft cells) increased as cells advanced up the villus (75). Subsequently, crypt-villus “zonation” was described using single cell transcriptomics and laser capture microscopy to dissect epithelial cell function at a single cell level along the crypt-villus vertical axis (134). The markers used to delineate these different zones along the crypt-villus axis were subsequently leveraged in an approach called “ClumpSeq”, which improves rare cell sampling with droplet-based sequencing small cell aggregates in lieu of single cells (135). Using that method, Manco et al reported Tuft-2 (immune) tuft cell transcripts at the villus tip, and Tuft-1 (neuronal) transcriptional signature toward the bottom of the villus. Since the intestinal epithelium is renewed by proliferation of transiently-amplifying progenitors near the crypt base which 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 (136).

Though best explored in the intestine, the ontogeny of tuft cell heterogeneity has also been explored 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 (104) and similarly suggested temporal regulation of tuft cell gene expression programs, perhaps analogous to the crypt-villus maturation model in the intestine. Importantly, this work noted similarity between early stage tuft cells and the “neuronal” signature identified in Haber et al, while the Tuft-2 signature, including *Il25*, was enriched at later stages. Such data further supports 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 lockstep 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 (where tuft

cell heterogeneity has also been described), it is tantalizing to consider how tuft cell heterogeneity may relate to microanatomic locations, such as proliferative “hillocks” from which basal cells were recently found to repopulate the injured trachea (83). Likewise, in the extrahepatic biliary tree, where tuft cell heterogeneity was also noted (O’Leary et al, in press), tuft cells expressed discrete transcriptional programs which may arise in response to unique luminal exposures in differing anatomic locations, such as in the fundus of the gallbladder, 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 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 (137) (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 numerous 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 (138), 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.

Differential 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 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- (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 tissue niches that is observed as heterogeneity provides clues to these questions. Specifically, we propose that all newly differentiated “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 drive maturing

tuft cells towards one or more dominant effector programs. For instance, tissue maturation cues specific to the small intestine may drive expression of SUCNR1 (which is relatively intestinal tissue-specific) as tuft cells ascend the villus (perhaps defined by spatial guides, such as trophocytes and telocytes (139)). Acquisition of SUCNR1 expression during villus ascension would be expected to coincide with increasing exposure to luminal succinate, thereby driving 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. When contemplating this paradigm, it is interesting to consider whether the particularly high expression of GNAT3 in nascent pancreatic tuft cells facilitates receptivity to environmental GPCR ligands that drive their mature effector program (104). The concentration of potential tuft cell ligands in luminal contents 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 towards 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 bi-stable switches controlling discrete on/off states based on relative availability of agonists or antagonists.

Also unexplored within the above paradigm is the function 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 simply 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 (140)? Potential alternate(140) roles for nascent tuft cells could be a poised ability to return to the crypt and stand in for Paneth cells to support the recovering stem cell niche after damage (141). If indeed nacent 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 (O’Leary et al, in press; (82)).

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 sentinals 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/growth factors from stromal or other niche cells, 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 towards therapeutic aims.

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Sidebars

Sidebar 1: Tuft cells in viral infection

There is emerging evidence that tuft cells may not just regulate responses to helminths and parasites, and impact antibacterial immunity, but are target cells in viral infection. In murine norovirus infection, small intestinal tuft cells are the cell source of viral entry, through viral particle engagement with the surface receptor CD300lf (142; 143). Tuft cells can be also be infected with rotavirus (131). In the mouse model of norovirus, tuft cell infection serves to reduce lamda interferon responses, promoting infection and creating a viral reservoir (144). However, tuft cells are not the target cell in human norovirus (145; 146), and the role of tuft cells in human viral infection remains unclear.

Sidebar 2: 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 (147). 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 decision about bulk ingestion by orchestrating behavioral responses (favoring “positive” tastes/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.

Sidebar 3: 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 provokes speculation that tuft cells could serve a peri-neuronal 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 (47–49; 70) and intestine (41). However, aside from functional evidence supporting interaction with trigeminal nerves in the upper airway (discussed above; (47–49)), 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 (41), but 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 (148), whereas tuft cells in the adjacent respiratory epithelium may plausibly require extended cytoplasmic processes to reach beyond the basement membrane to nerves below.

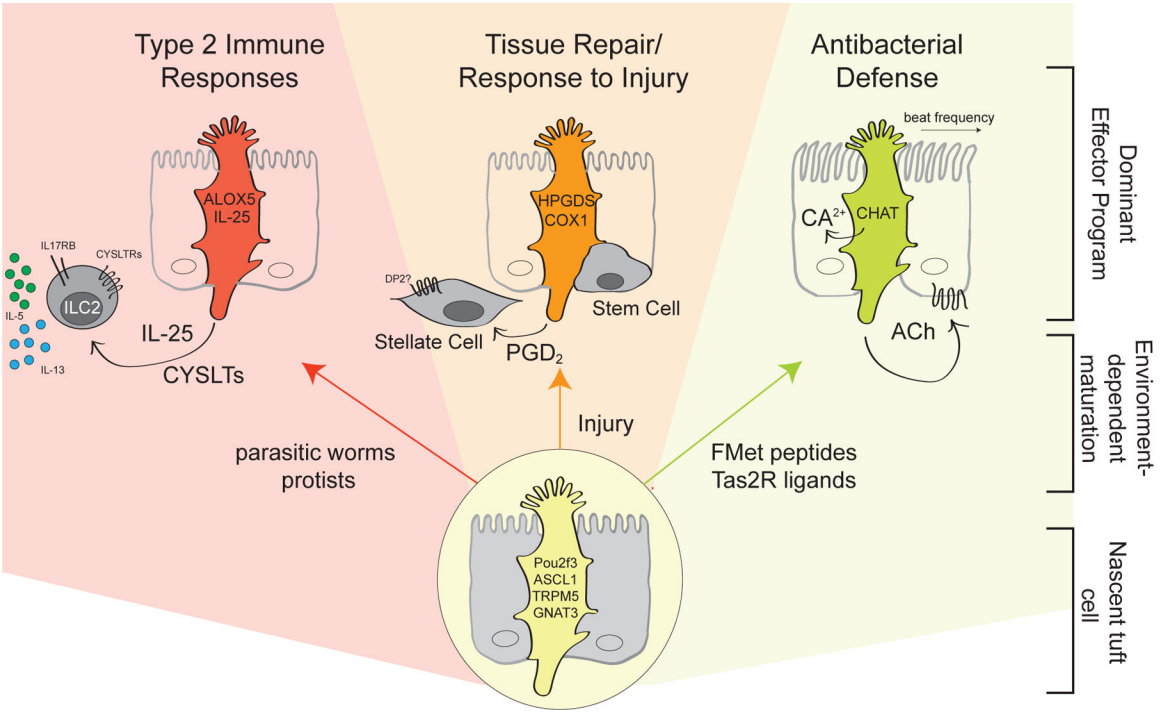

Figure 1. Major functional outputs of tuft cells.

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. 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 IL-25 and cysteinyl leukotrienes was critical for anti-helminth responses, while IL-25 alone promoted adaptive responses to protist colonization downstream of succinate sensing (recently reviewed in (19)). Roles for tuft cells in tissue regeneration or response to injury have been demonstrated in models of colitis and intestinal stem cell loss (14; 128; 141) and in pancreatitis-induced pancreatic cancer (31). In the injured pancreatic duct, tuft cell PGD_2 repressed inflammatory gene expression in stromal cells and slowed tumorigenesis. Antimicrobial defensive function is best characterized in the conducting airways, where bacterial-derived formylated peptides (FMet) or TAS2R ligands induced tuft cell-mediated production of acetylcholine (ACh) and/or lateral calcium release, increasing ciliary beat frequency of neighboring epithelial cells and promoting antimicrobial peptide release (53; 54). Forthcoming work also supports antibacterial roles in the small intestine, via vomeronasal receptor signaling (29), 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 (i.e.: presence or absence of activating ligands such as succinate or FMet peptides), promoting environmental-driven maturation of effector functions from nascent tuft cells.

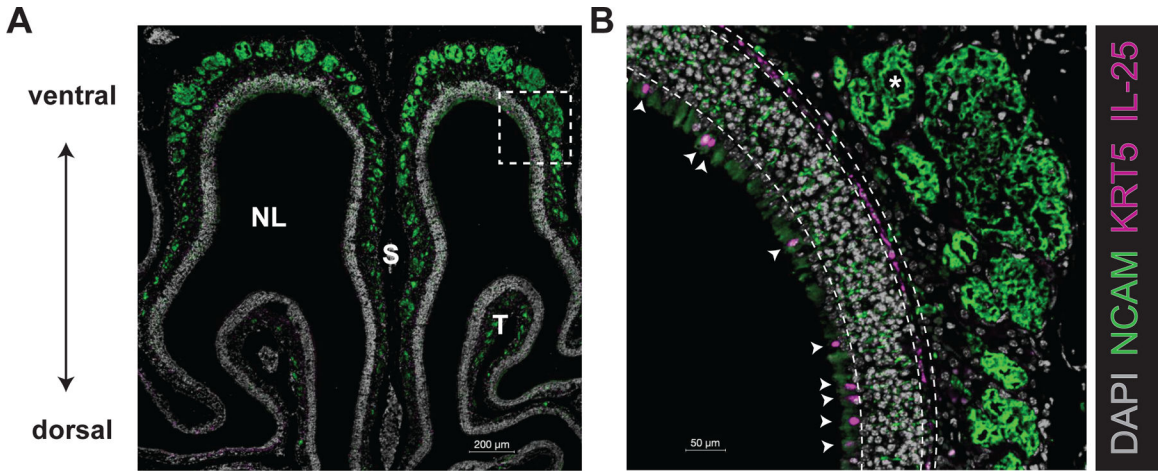

Figure 2. IL-25+ tuft cells in olfactory epithelium.

Neuroepithelial tissues have critical roles in chemosensation that directs attractive and aversive behaviors. Tuft cells in the olfactory epithelium, 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. NL = nasal lumen, T = turbinate, S = septum. **(B)** Inset from (A). OE tuft cells, aka “microvillus cells,” are IL-25+ (red) cells in the apical epithelial layer (marked by white arrow heads), above and in direct contact with olfactory sensory neurons (OSNs). OSNs are observed as a pseudostratified array of nuclei (DAPI, white) outlined by neural cell adhesion molecule 1 (NCAM, green) processes/cytoplasm, denoted between white dashed lines. A thin layer of keratin 5 (KRT5, red)-stained horizontal basal cells are also delineated by white dashed line. An NCAM+ nerve fiber is denoted with a white asterisk.

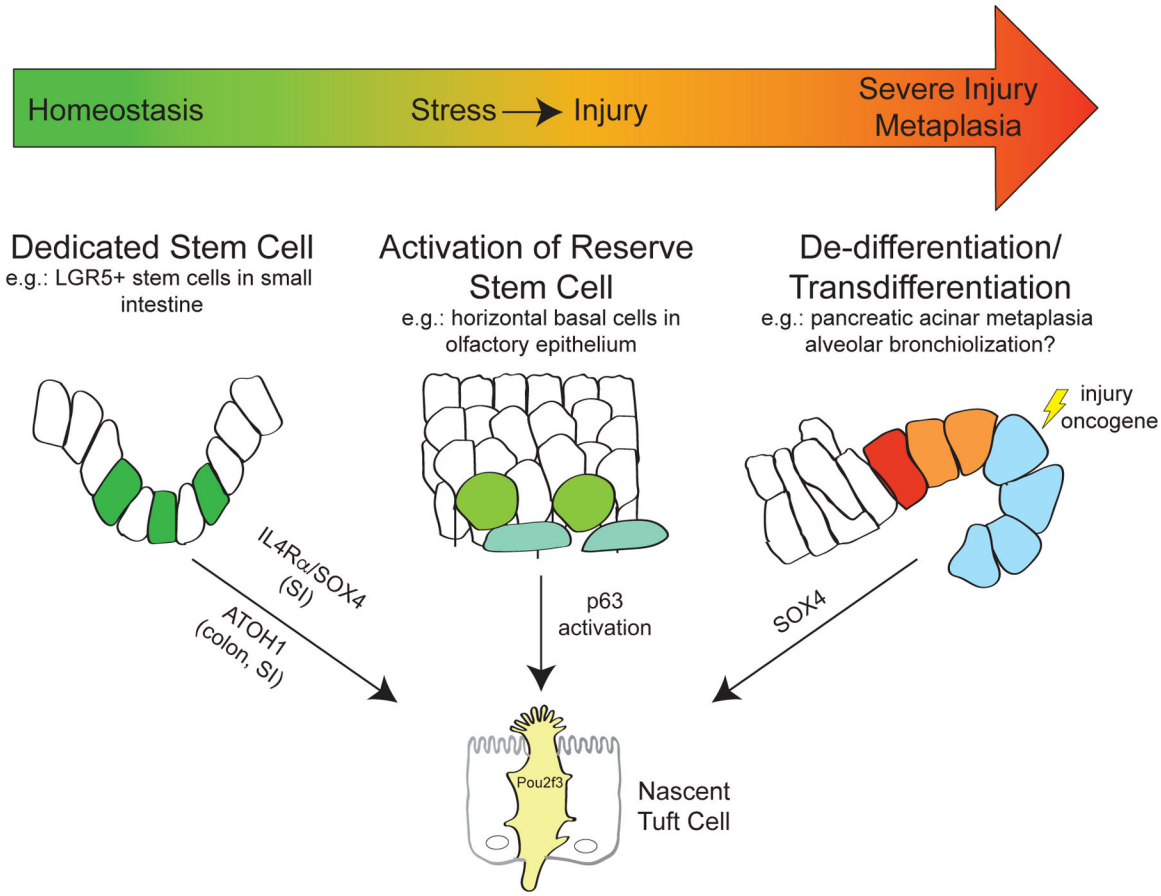

Figure 3. Tuft cell differentiation at homeostasis and in injury.

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. In the small intestine, 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 can be traced to globose basal cells. Local epithelial progenitors remain to be identified in some tuft cell-containing tissues (e.g.: extrahepatic biliary tree). 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 olfactory epithelium, where otherwise quiescent horizontal basal cells are activated to renew all OE cells including tuft cells (89–91). 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 (103; 104) indicates that under severe injury, fully differentiated acinar cells de-differentiate or transdifferentiate into tuft cells, passing through a mucinous intermediate. Whether this could be the process driving 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.

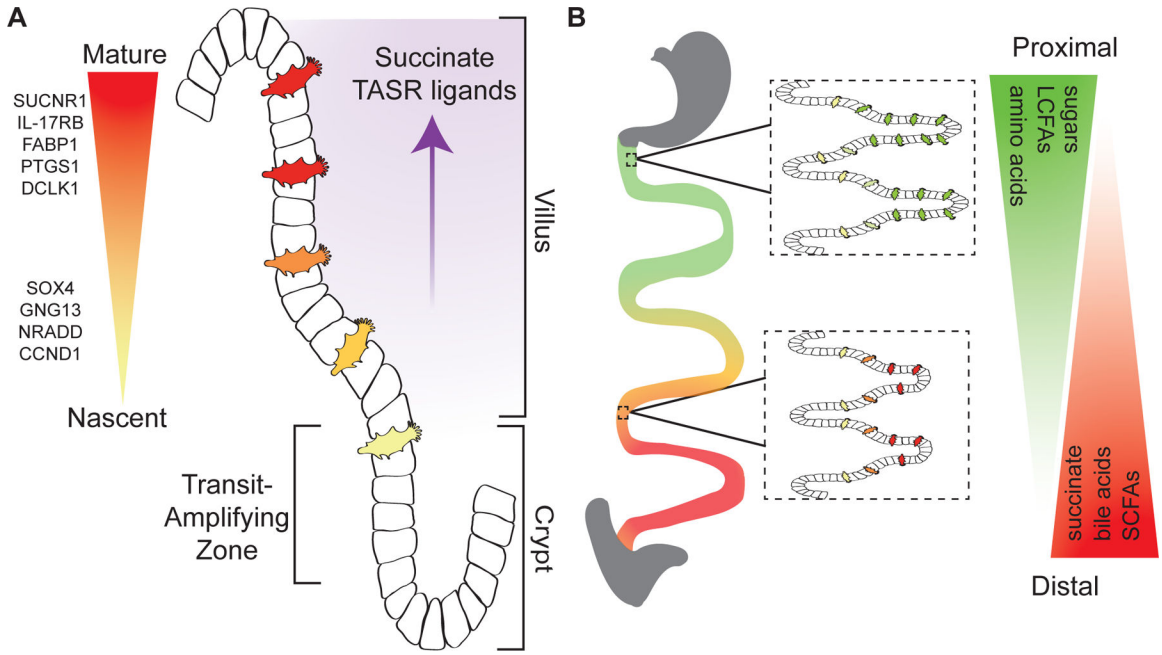

Figure 4. Heterogeneity of tuft cells across space and time.

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. In the small intestine **(A)** tuft cell gene expression profiles change along on the crypt-villus axis, which is concordant with cellular age and increasing exposure to luminal contents, including known tuft cell ligands like succinate. Many transcripts associated with immune function were enriched in tuft cells toward the villus tip (135), while transcripts previously associated with a “neuronal” phenotype were associated with physical position (peri-cryptal) or cellular age (newly differentiated), comprising 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 (i.e.: 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.