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Review

Tuft cells: From the mucosa to the thymus

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ABSTRACT

Tuft cells are epithelial chemosensory cells with unique morphological and molecular characteristics, the most noticeable of which is a tuft of long and thick microvilli on their apical side, as well as expression of a very distinct set of genes, including genes encoding various members of the taste transduction machinery and pro-inflammatory cyclooxygenases. Initially discovered in rat trachea, tuft cells were gradually identified in various mucosal tissues, and later also in non-mucosal tissues, most recent of which is the thymus.

Although tuft cells were discovered more than 60 years ago, their functions in the various tissues remained a mystery until recent years. Today, tuft cells are thought to function as sensors of various types of chemical signals, to which they respond by secretion of diverse biological mediators such as IL25 or acetylcholine. Intestinal tuft cells were also shown to mediate type 2 immunity against parasites.

Here, we review the current knowledge on tuft cell characteristics, development and heterogeneity, discuss their potential functions and explore the possible implications and significance of their discovery in the thymus.

1. Introduction

Tuft cells are epithelial chemosensory cells with unique morphological and molecular characteristics, the most noticeable of which is a tuft of long and thick microvilli on their apical side (Fig. 1). Although they were discovered more than 60 years ago, their functional role has remained a complete mystery till recently.

While tuft cells were originally identified in the rat trachea [1] and mouse stomach [2], they were later found to be an integral part of many other (mainly mucosal) mammalian tissues, including the respiratory tract, small and large intestine, salivary gland, gallbladder, pancreatic duct, auditory tube and urethra [3–20]. For this reason, they are often referred to by several other names, including, brush, microvillar, caveolated, fibrillovesicular, multivesicular or solitary chemosensory cells (SCC).

In general, tuft cells are thought to function as sensors of various types of chemical signals [5,21,22], to which they respond by secretion of diverse biological mediators, such as IL-25 or acetylcholine [23–26] (Fig. 2). For instance, intestinal tuft cells have been shown to play a key role in parasite sensing and subsequent initiation of an effective anti-parasitic response via secretion of IL-25 [23–25], whereas tuft cells of the trachea were implicated in the regulation of respiration [27]. Interestingly, tuft cells were recently found also in the thymus [28,29], a non-mucosal, primary lymphoid organ which is essential for the development and maturation of T cells. This suggests that tuft cells can also be found in non-mucosal and non-polarized tissues, where they can play additional and/or yet-undefined functional roles.

In this review, we will describe the current knowledge regarding tuft cells, their development and heterogeneity, discuss their potential functions and explore the possible implications and significance of their discovery in the thymus.

2. Tuft cell characteristics

Most tuft cells are characterized by a long cylindrical or flask-shaped body, which is wider around the nucleus and narrows toward the apical end or toward both apical and basal ends [30]. However, the most notable feature that characterizes virtually all types of tuft cells is a layer of tuft- or brush-looking microvilli found on the apical side of the cell and penetrating the lumen (Fig. 1). These microvilli are typically connected to a rich network of microfilaments and microtubules, which can extend deep into the cell's cytoplasm [31–33] (Fig. 1). Interestingly, some tuft cells were also found to possess thin lateral microvilli called cytospinules, which project from the tuft cell and penetrate neighboring epithelial cells (Fig. 1) [34–36]. These lateral projections were shown to make physical contact with the nucleus of neighboring cells, where their tips are usually wrapped by the neighbor cell's nuclear membrane [37]. This form of contact between cells is quite unusual and was suggested to play a role in the transfer of cargo, perhaps even genetic material.

The tuft apical microvilli and their rootlets in the apical cytoplasm contain F-actin microfilaments, and can therefore be stained by antibodies specific to various cytoskeletal proteins including actin, fimbrin or villin [38,39]. Tuft cells also contain two types of intermediate

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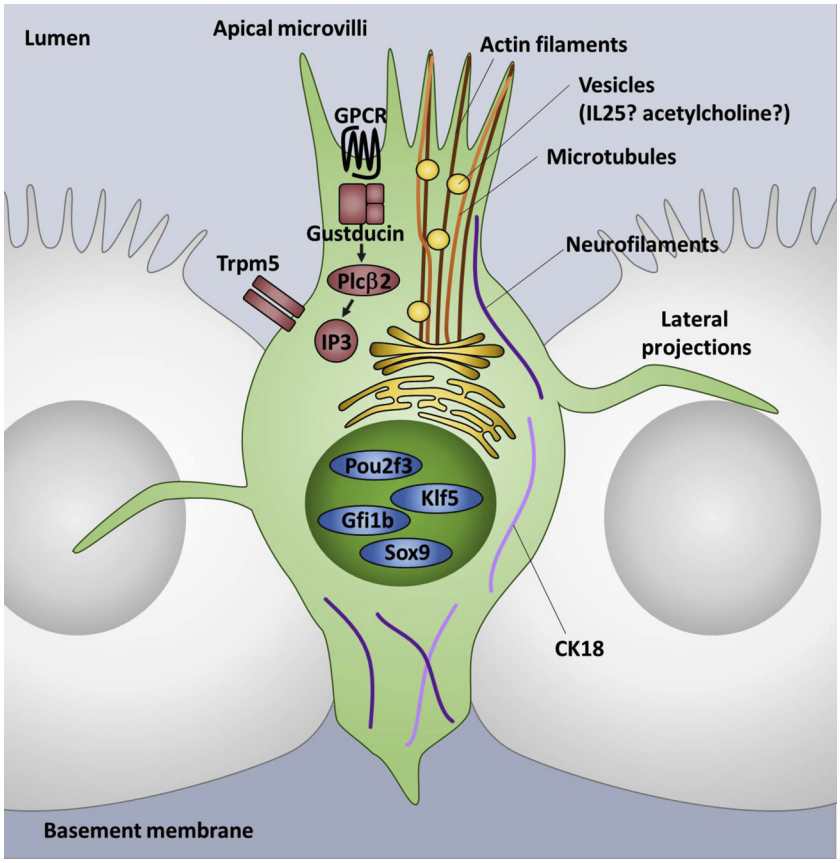

Fig. 1. Tuft cells characteristics. Tuft cells are distributed between other epithelial cells in the lining of mucosal tissues. On their apical side is a thick brush of microvilli, which are usually longer and wider than those of neighboring cells. Lateral projections extend from the cell and penetrate neighboring cells, in which they contact the nuclear membrane. In yellow – a long, vertical network of microtubules and microfilaments extends from the microvilli deep into the cytoplasm and reaches the endoplasmic reticulum. Vesicles, which likely contain secreted molecules, are spread along the filamentous network. In purple – two types of intermediate filaments are found in tuft cells, cytokeratins (CK) 8 and 18, and neurofilaments. While neurofilaments are localized to the apical cytoplasm between the actin filaments and microtubules, cytokeratin 18 is found in the perinuclear region or spread in the entire cytoplasm, and both co-localize at the basolateral margins. In Blue – several characteristic transcription factors are expressed by tuft cells, including Pou2f3, Gfi1b, Sox9 and Klf5. In red – tuft cells express components of the taste transduction machinery, including the specific G-protein gustducin, Phospholipase C Beta 2 (Plcb2) which hydrolyzes phosphatidylinositol 4,5-bisphosphate to the second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol, and the cation channel Trpm5. This pathway is activated in tuft cells of different tissues by various G-protein coupled receptors (GPCRs), including Sucnr1, taste receptors and $CysLT_3R$.

filaments including epithelia-specific Cytokeratin-18 [39], and surprisingly also neurofilaments, which characterize mature neurons [40]. These filaments were shown to have a compartmentalized distribution, with neurofilaments localized to the apical cytoplasm between the F-actin filaments and microtubules, cytokeratin 18 in the perinuclear region or spread in the entire cytoplasm, and both co-localize at the basolateral margins [40,41]. Ankyrin, known to link the cytoskeleton to membranal proteins, is localized in tuft cells mainly near the basolateral membrane [41]. Doublecortin-like kinase 1 (Dclk1), which might be associated with microtubules polymerization, was also identified as a tuft cells marker [42,43]. More recently, an actin-binding protein called girdin [44,45], was shown to be a highly specific marker for intestinal tuft cells when phosphorylated on its tyrosine 1798 [46]. It is however unclear, whether it can be used as a specific tuft cell marker also in other tissues.

In addition to the above-mentioned structural markers, tuft cells are also characterized by a specific expression of various genes, some of which may be critical for mediating their functional role(s). For instance, most tuft cells express various components of the taste reception signaling machinery, including gustducin [5,47], Transient receptor potential cation channel subfamily M member 5 (Trpm5) [48], phospholipase C β (PLCβ) [49] and various types of taste receptors [50] (Fig. 1), highlighting the similarities between tuft cells and taste buds cells, suggesting that they may be involved in taste chemosensation.

Furthermore, tuft cells also express various components of the biosynthesis pathway of prostaglandins and leukotrienes such as hematopoietic leukotriene D synthase (Hpgds) and cyclooxygenases (Ptgs1) [51,52] or fatty acid metabolism-related proteins, such as liver fatty acid binding protein (L-FABP) [53], suggesting that they may produce and secrete prostaglandins and leukotrienes, mediators that regulate many physiological processes including inflammation. Moreover, some types of tuft cells were also reported to express various components of the nitric oxide synthesis pathway such as nNOS and NADP-linked G6PD [54] or blood pressure homeostasis-related proteins, such as renin, angiotensin and several electrolyte transporters [51,55–57]. Tuft cells also express several neuronal pre- and post-synaptic proteins, including choline acetyltransferase (ChAT), which is essential for the synthesis of the neuromodulator – acetylcholine [51,58,59].

Finally, one of the key factors that is abundantly and specifically expressed by tuft cells is IL25 (and/or Thymic stromal lymphopoietin, TSLP), an important cytokine involved in initiation of the type-2 immune response [23–25].

However, some of these molecular markers are either tissue and/or context specific or are not exclusive to tuft cells. For example, nitric oxide (NO) synthesis pathway components are expressed by tuft cells in the stomach, but not by intestinal tuft cells [60]. Therefore, as suggested by Gerbe et al. [61], a combination of various tuft cell markers, together with morphological features, is probably the most accurate and specific way to identify tuft cells in the specific tissue.

In addition, the heterogeneity of tuft cells seems to be further multiplied by different types of tuft cells even within the same type of tissue. Recently, Haber et al. [62] performed single cell RNA seq analysis of small intestine epithelial cells, highlighting 2 different clusters of tuft cell progenitors and 2 distinct clusters of mature tuft cells. When focusing on the two mature tuft cell clusters, they observed that one of them (named tuft 2) was enriched for immune-related genes, whereas the other (named tuft 1) expressed a neuronal development gene signature. In addition, although both tuft 1 and tuft 2 expressed IL25 and the receptors for IL25, IL4 and IL13, only tuft 2 expressed TSLP and proliferated in response to infection by the helminth *H. polygyrus*, suggesting that the two clusters might represent different functional types of tuft cells. Furthermore, a recent study by Montoro et al. [63] found that tuft cells of the trachea are similarly divided into two types of mature cells, with tuft-1 expressing genes associated with taste transduction such as *Plcb* and *Gnat3*, and tuft-2 expressing immune-related genes such as *Alox5ap* and *Ptprc*.

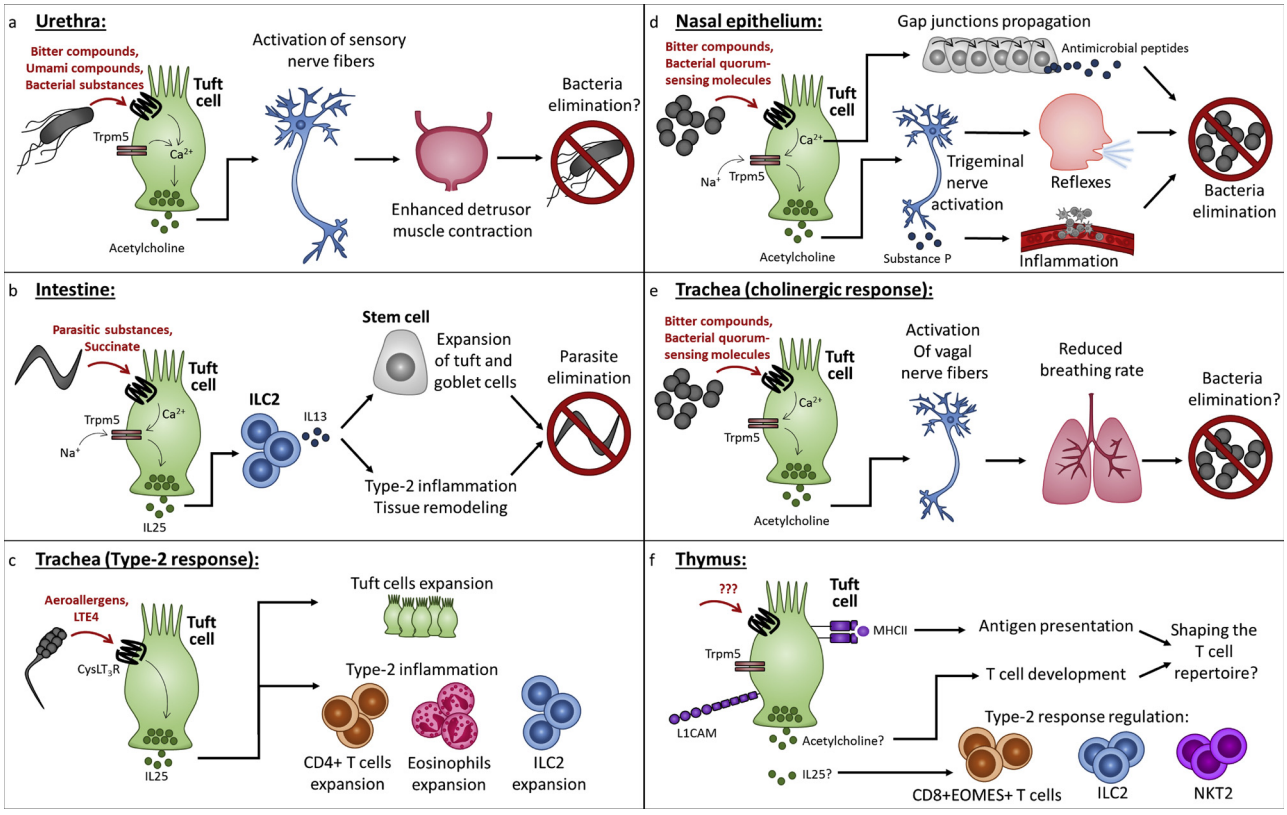

Fig. 2. Tuft cells functions. (a) Urethra: Heat-inactivated *e. coli*, and bitter and umami tastants, cause an increase in intracellular Ca^{2+} in cholinergic, Tas2R108- and Tas1R1-Tas1R3 expressing brush cells. Even though Trpm5 is a nonspecific cation channel usually activated by Ca^{2+}, in these cells it is required for Ca^{2+} increase. Ca^{2+} triggers acetylcholine secretion, resulting in detrusor muscle reflex activation and bladder emptying, likely through sensory nerve fibers which are found in contact with the tuft cells. Flushing through micturition is a known protective mechanism in the urinary tract, and therefore tuft cells might be activating such a response to eliminate bacteria. (b) Intestine: Tuft cells in the intestine sense parasites through Sucnr1, and perhaps other receptors, and respond in a Trpm-dependent manner by secreting IL-25. ILC2 cells expressing IL-25R are then activated and secrete IL-13, which acts both by recruitment of type-2 related cells such as eosinophils, as well as by altering the differentiation program of crypt epithelial stem cells to generate more tuft and goblet cells. This response is important for parasite elimination. (c) Trachea (type-2 response): A subset of the tracheal tuft cells express the leukotriene E4 (LTE4) receptor $CysLT_3R$, and they are also known to express components of the leukotriene generation machinery. Tracheal tuft cells respond to both aeroallergens such as *Alternaria*, which are known to cause LTE4 elevation in the epithelium, and to LTE4 itself, by secretion of IL-25. This cytokine is necessary for both expansion of the tuft cells, as well as for activation of a type-2 immune response which includes a general expansion of the CD45+ compartment, and specifically ILC2 cells, CD4+ T cells and eosinophils. (d) Nasal epithelium: T2R bitter taste receptors-expressing tuft cells respond to both bitter compounds, as well as bacterial quorum-sensing molecules such as acyl-homoserine lactones, by elevation of intracellular Ca^{2+}, which leads to Trpm5-dependent membrane depolarization and secretion of acetylcholine. This results in the activation of trigeminal nerve fibers expressing acetylcholine receptors, thus activating a protective reflex of breathing depression. Trigeminal nerve activation is known to also promote local inflammation through release of CGRP and substance P. Finally, the Ca^{2+} wave in activated tuft cells of the human nasal epithelium propagates through gap junctions to neighboring epithelial cells, causing secretion of antimicrobial peptides into the mucus. Together, these mechanisms can promote elimination of bacteria. (e) Trachea (cholinergic response): Tracheal tuft cells sense bitter tastants through T2R receptors, as well as bacterial quorum-sensing molecules such as 3-OxoC(12)-HSL (known to be produced by *Pseudomonas aeruginosa*). This activates the downstream taste-specific signaling cascade which results in secretion of acetylcholine. Vagal nerve fibers which innervate the tuft cells respond to acetylcholine by causing a reflex of reduced breathing rate, likely aiding in bacteria elimination. (f) Thymus: Thymic tuft cells express T2R bitter taste receptors and components of their downstream signaling cascade, including Trpm5, however the signals to which they respond are currently unknown. They express MHCII molecules and likely participate in tolerance induction by negative selection of T cells. Due to their expression of ChAT, it is possible that thymic tuft cells generate and secrete acetylcholine, which was shown to regulate T-cell development. They also express IL-25, which is the most likely candidate through which they control thymic populations of type-2 related cells such as ILC2, NKT2 and EOMES+CD8+T cells.

In regards to their distribution, in most tissues tuft cells seem to be distributed in a sporadic and solitary manner, and are not seen near to other tuft cells [22,46]. Their frequency seems to vary greatly between different organs. While in the major pancreatic duct they account for over 20% of all epithelial cells [5], in the respiratory tract they account for 1–10% [9,64–67], in the stomach for 2% [43], and in the intestine for only ~0.4–1% of the local epithelial cell population [52,68].

In the thymus, the recently discovered tuft cells are found strictly in the medulla, where they are clustered in close proximity to terminally differentiated medullary thymic epithelial cells (mTECs) and account for 5–10% of total mTECs [28,29,69,70]. Importantly, thymic tuft cells were shown to express many of the tuft cells markers mentioned above such as Ptgs, Gfi1b, ChAT, Dclk1, cytokeratin 18 and 8, villin, Pou domain class 2 transcription factor 3 (*Pou2f3*), as well as components of the taste receptor signaling machinery including gustducin, Plcβ2, Trpm5 and several T2R family receptors such as Tas2r131, Tas2r143/135/126 and others [28,29,69–71].

On the other hand, an RNA seq analysis showed that thymic tuft cells have the most distinct and differential gene expression signature from all other tuft cells [72]. Specifically, unlike other types of tuft cells, thymic tuft cells are characterized by expression of a surface molecule L1 cell adhesion molecule (L1CAM) or by relatively high expression of molecules involved in antigen presentation MHCII and CD74 [28,29].

The morphological features of thymic tuft cells are less clear than in other tissues, partly because the tissue epithelium organization differs from that of mucosal tissues, with no clear orientation of an apical and a basal end, but it was observed that they appear bulbous, with a long

narrow process or lateral microvilli extending between neighboring epithelial cells [28,29,73]. However, additional investigation of their morphological characteristics and tissue distribution in relation to other cells in the thymus is required.

3. Tuft cell development

During ontogeny tuft cells seem to appear only after birth or in the later stages of embryonic development. For instance, tuft cells in the rat submandibular gland were found to appear only 17 days after birth [74], while Dclk1^{+} tuft cells in the mouse intestine are detected only 1 week after birth [43,52,75]. Similarly, thymic tuft cells are absent in the embryonic thymus and appear only several days after birth.

The origin of tuft cell was most extensively studied in the gastrointestinal (GI) tract. Tuft cell progenitors were first identified in 1979, when Tsubounchi et al. performed 3H-thymidine incorporation experiments and found that the lower portion of the crypt of the descending colon contains putative tuft cell progenitors [15]. Subsequent mutagenesis-based clonal analysis by Bjerknes et al. demonstrated that tuft cells share a common ancestor with enterocytes and goblet cells [76]. Later, lineage tracing experiments demonstrated that this cellular origin is the Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5)-expressing columnar stem cells at the base of the crypt [52]. It was also shown that the mature tuft cells outside of the crypt are post mitotic, constantly replenished and have a short life span of approximately 1 week [52].

Attempting to define the molecular requirements for tuft development in the GI tract, Gerbe et al. (2011) tested whether tuft cell require the secretory lineage-specific transcription factor Atonal homolog 1 (Atoh1) for their specification and/or differentiation. Using a tamoxifen-inducible villin-driven cre recombinase, they deleted *Atoh1* by daily IP injections of tamoxifen and found that the small intestine was consequently void of tuft cells as well as of other secretory cells whose development depends on Atoh1 including goblet, enteroendocrine and Paneth cells. However, in contrast to these findings Bjerknes et al. demonstrated that rosa26^{creERT2} driven deletion of *Atoh1* using gavage administration of tamoxifen results in elimination of all secretory cells in the small intestine but not of tuft cells, even though Atoh1 was deleted in them as well [75]. The controversy between the two studies was proposed to result from toxicity of the treatment in the first study. However, several findings such as weak Atoh1-EGFP signal detected in immature tuft cells found lower in the crypt, expression of LacZ in tuft cells following lineage tracing with Atoh1^{creERT2}xRosa26-LacZ mice, or the fact that tuft cells are not present in embryonic E18.5 Atoh1$^{-/-}$ proximal intestine, might support an alternative explanation, in which in the second study Atoh1 was not deleted in tuft cells progenitors due to the lower tamoxifen concentration, which only affected the mature tuft cells (that are no longer Atoh1 dependent), thus allowing mature tuft cell differentiation. Since markers for tuft cell progenitors were not found yet, this hypothesis is yet to be validated. Another model, proposed in a recent review [3], suggests that embryonic tuft cells, which develop in a pathogen-free environment, are *Atoh1*-dependent, while the mature tuft cells are *Atoh1*-independent, possibly since they arise from different progenitors which might be activated by exposure to parasites through IL-4 signaling and Sox4 expression.

Interestingly, additional transcriptional factors such as Neurogenin 3 (*Neurog3*), SRY-box containing gene 9 (*Sox9*), or Growth factor independent 1 (*Gfi1*), which are required for the development of enteroendocrine cells, Paneth and/or goblet cells respectively are all dispensable for the development of intestinal tuft cells [52].

Importantly, the key master regulator of tuft cells development seems to be *Pou2f3* (Fig. 1), which was found to be critical for the development of tuft cells in the intestine, nasal respiratory epithelium as well as in the thymus [29,77–79]. Interestingly, in addition to its key role for tuft cells development, *Pou2f3* is also essential for the generation of sweet, umami, and bitter taste cells [80], further highlighting similarities between these two cells types.

The origin and development of tuft cells in the thymus is still poorly understood and seems to be quite different from the development of tuft cells in other tissues. Specifically, two recent studies suggested that thymic tuft cells appear only after birth and seem to be directly derived from mature MHCII^{hi} mTECs. This notion is mainly supported by two independent in vivo models for lineage tracing including the inducible *Aire*-CreERT ZsGreen reporter [29] and the *Csnb*.Cre-tdTomato reporter [79], which both demonstrate that thymic tuft cells have a previous history of mature MHCII^{hi} mTECs cells. However, it is possible that an alternative (and still undefined) developmental pathway exists. Such possibility is supported by several interesting observations; First, treatment of the BALB/cByJ × *Aire*^{DTR} C57BL/6 F_1 mice with diphtheria toxin leads to a rather moderate (< 50%) (yet significant) reduction in thymic tuft cells, suggesting that a large fraction of thymic tuft cells is not derived from Aire-expressing mTECs. Second, Aire deficiency was found to have virtually no impact on the development of thymic tuft cells, while it had a profound impact on the development of other terminally differentiated Keratin-10-expressing mTEC subsets that are derived from Aire^{+} mTECs [79]. Third, single cell RNA seq and ChIP seq analyses demonstrate that tuft cells and Aire^{+} mTECs are both transcriptionally and epigenetically very distant with no intermediate population that could explain the developmental transition from the Aire^{+} mTECs to the tuft cells. Therefore, further experimental work is needed to better elucidate the exact developmental program(s) of thymic tuft cells.

4. Tuft cells in disease

Although tuft cells have been implicated in various pathologies, their exact role in health and disease is still incompletely understood and requires further investigation.

For instance, tuft cells were suggested to be involved in diseases such as inflammatory bowel disease (IBD) or infections by *Clostridium difficile* (*C. difficile*). Specifically, the symptoms of these diseases were shown to inversely correlate with the levels of IL25, the key cytokine that is mainly produced by tuft cells. Similarly, biopsies of intestinal mucosa from patients with ulcerative colitis or Crohn's disease [81] revealed that they contain fewer IL25-expressing cells. Therefore, these data suggest that intestinal tuft cells may play an important role in maintaining intestinal homeostasis and/or in prevention of local inflammation. This hypothesis is further supported by data demonstrating that restoring IL25 levels in the mouse model of *C. difficile*-induced colitis reduces the symptoms and increases survival through eosinophil-dependent protection of the gut barrier [82].

In other cases, however, tuft cells may not necessarily play protective roles. Specifically, tuft cells were found to increase in numbers following stomach inflammation, hyperplasia and metaplasia in the GI tract [43] or in chronic rhinosinusitis with nasal polyps (CRSwNP [83]). In the human airway epithelium, increased tuft cells abundance was shown at the expense of tracheal ciliated cells in a patient with immotile cilia syndrome [84], and in lung biopsies of an infant with bilateral pneumothoraxes and respiratory stress [85]. However, in both cases it is not clear whether the increased tuft cell numbers merely reflect the symptoms or actually play a causative role.

Interestingly, the tuft cell marker Dclk1 was shown in recent years to be expressed in a variety of tumors, and knock-out studies have shown that it is functionally important for tumor growth or progression. For instance, Dclk1^{+} cells are overrepresented in ~75% of human primary colorectal cancers and colorectal adenocarcinomas [86] and exceptionally high levels of Dclk1^{+} cells were correlated with lower survival. Similarly, Dclk1^{+} cells were also overrepresented in neuroendocrine tumors of the mammary gland and the rectum [87,88], and in esophageal cancer [89]. Suppression of the Dclk1 gene caused a growth arrest of the colorectal xenograph [90], increased apoptosis in neuroblastomas [91] and growth arrest in hepatocellular carcinomas

[92]. A study conducted by Nakanishi et al. in 2013 demonstrated that Dclk1$^+$ cells represent the tumor stem cells. Combining this model with diphtheria toxin mediated deletion of Dclk1$^+$ cells led to a significant regression of established tumors [93].

Furthermore, the expression of Dclk1 in various cancers and its effect on the expression of various oncogenes such as c-myc and Kras [90,94] highlights Dclk1 as a potential therapeutic target. Indeed, genetic and pharmacological targeting of Dclk1 in Kras mutated mouse models of pancreatic cancer led to significant inhibition in pancreatic tumorigenesis [95]. Similarly, recent studies suggested that a direct relationship between Dclk1 and Cox2, both tuft cell markers, exist also in pancreatic cancer. The expression of both proteins increases as the tumor progresses, and Cox2 was also described to be a Kras downstream effector [96]. Treatment with a cox inhibitor, Licofelone, led to a significant decrease in Dclk1 expression and pancreatic cancer stem cells [97].

It should be stressed however, that most studies involving Dclk1 and cancer have focused on Dclk1 alone, leaving the question regarding the exact identity of the Dclk1$^+$ cells open. The fact that tuft cells are the predominant population expressing Dclk1 under homeostatic conditions, and the connection between Dclk1 and Cox2 both in tuft cells and in cancer progression makes them a likely suspect. However, whether tuft cells are indeed directly responsible for various types of tumors needs to be experimentally validated in future studies. In line with this, Bailey et al. [98], did show that Dclk1$^+$ cells with tuft-like characteristics were present in murine pancreatic intraepithelial neoplasia. These cells were shown to exist under homeostasis conditions as progenitor cells that are important in regeneration, but following pancreatic injury, can become a powerful source of cancer initiating cells.

5. Tuft cell functions

As already mentioned above, although tuft cells have been identified more than 60 years ago, their functional role remained poorly understood for a very long time.

One of the key breakthroughs in understanding the potential functional role of tuft cells was the realization that they share several unusual features with the chemosensory cells in taste buds [5,17], including the expression of various taste receptors and the taste transduction signaling machinery. This suggested that tuft cells may function as chemoreceptive cells capable of sensing various chemical signals in their environment. However, which signals, and what molecules they are able to sense, still remains poorly understood. Nevertheless, increasing body of evidence suggests that tuft cells can be activated via the taste receptor machinery, as well as via various small molecules, such as succinate, aeroallergens and Leukotriene E4 (LTE4) [72,99–101] (Fig. 2).

Moreover, the fact that some tuft cells contain large vesicles near the apical membrane [21,102] suggests that tuft cells might have a secretory role. Indeed, tuft cells are known to express various secretory molecules (or machinery required for their synthesis) with important biological functions, including IL25, TSLP, acetylcholine, leukotriens, prostaglandins, endogenous opioids, renin or nitric oxide.

Based on these facts, tuft cells seem to function as sensors of possible danger signals to which they respond by secretion of various biologically active mediators. However, the specific signals they sense and the specific mediators they secrete seem to be tissue- and context-dependent.

For instance, tuft cells in the urethra [26] were found to respond to bitter compounds by activation of the taste receptor signaling cascade, resulting in a subsequent rise in intracellular Ca^{2+} and release of acetylcholine. This in turn is thought to activate various cells in their proximity, including sensory nerve fibers, eventually leading to bladder detrusor reflex accompanied by enhanced bladder emptying. Since some bacterial products are known to activate bitter taste receptors, it was hypothesized that this pathway might be involved in antibacterial defense (Fig. 2a).

More recent studies demonstrate that mouse intestinal tuft cells are activated by parasitic infections, to which they respond by secreting IL25 (which they already constitutively express under homeostasis). Since IL25 is the key activator of innate lymphoid cells type 2 (ILC2), this in turn results in the initiation and amplification of the type-2 cytokine response, characterized by secretion of various key cytokines from the ILC2 cells, including IL13. This subsequently promotes goblet cell hyperplasia, eosinophil recruitment and activation, ultimately resulting in effective parasitic clearance. The secretion of type-2 cytokines by ILC2 cells also enhances tuft cells differentiation in the crypt, thus causing tuft cells hyperplasia, leading to enhanced IL25 secretion, and thereby initiating a feed-forward loop [23–25]. More recently, the differentiation of intestinal epithelial stem cells (IECs) into tuft cells following parasitic infection was shown to require their interaction with Th2 cells [63], suggesting that Th2 activation is part of this feed-forward loop. The activation of intestinal tuft cells was shown to be induced by a metabolite succinate, which is produced by protozoa and binds to its specific receptor Sucnr1 on tuft cells [72,99,100] (Fig. 2b). Interestingly, this G protein coupled receptor and the G-protein gustducin were required for tuft cells hyperplasia and type 2 response activation triggered by protist, but not by helminth infection, while the downstream cation channel Trpm5 was required in both cases. Therefore, it is likely that additional metabolites or other molecules are able to activate the same downstream signaling pathway. Some effects of this pathway were even shown to last long after the succinate trigger was removed, and activation of the pathway rendered mice more resistant to future infection.

Furthermore, the role of intestinal tuft cells may also be critical for local tissue regeneration (which may occur as a result of parasitic infection). Specifically, irradiation mediated injury of mouse models with either intestinal epithelium-specific Dclk1 deficiency or Dclk1 overexpression demonstrated that Dclk1 + cells mediate tissue recovery and promote intestinal epithelium survival through maintenance of epithelial barriers [103–105]. Moreover the protective role of tuft cells is further illustrated in IBD mouse models of Dextran Sulfate Sodium (DSS) induced colitis [106], where specific deletion of Dclk1 in intestinal epithelial cells (IEC) reduced their proliferation following DSS treatment and resulted in increased gut permeability and elevated levels of IL1b and IL17.

It remains unclear how exactly tuft cells, which are considered post-mitotic and differentiated cells, can promote tissue regeneration; interestingly, several studies have shown that Dclk1+ cells have stem-cells and self-renewal abilities [103,107,108], which might also explain their involvement in malignancies. While it is possible that Dclk1 might be expressed by other, non-tuft stem cells, Chandrakesan et al. [105] suggested that regeneration is mediated by paracrine signaling from tuft cells to neighboring intestinal stem cells. Another hypothesis suggests that under stress conditions which cause stem cells depletion, tuft cells, like other post-mitotic intestinal epithelial cells, can de-differentiate into stem cells to replenish the epithelial niche [3].

Together, these findings suggest that intestinal tuft cells play a critical role in protecting the gut from various hazards including parasitic infection and/or local injury.

Interestingly, tuft cells of the airways were recently [101] also shown to activate a type 2 immune response, but through a different mechanism than described above for the intestinal tuft cells. Specifically, Bankova et al. demonstrated that tuft cells in the trachea can expand in clusters in response to various aeroallergens or LTE4. This mechanism was shown to require the activation of the cysteinyl leukotriene 3 receptor (CysLT3R) receptor, and led to lung inflammation, eosinophilia, ILC2 cell expansion and CD4 T cell recruitment. While most of these effects were dependent on IL-25 secretion but not on Signal transducer and activator of transcription 6 (Stat6)/IL-13 signaling, some were IL-25-independent or Stat6-dependent, indicating that several parallel pathways in tracheal tuft cells transduce signals

from the CysLT3R (Fig. 2c).

In the nasal cavity, the rodent tuft cell-like SCCs were shown to respond to certain irritants by activating a trigeminal nerve reflex, known to be required for expulsion of irritants by breathing regulation and inflammation induction [109–111]. The signaling is mediated by activation of SCCs T2R bitter taste receptors and the TRPM5-related downstream signaling cascade, which, through acetylcholine secretion, activates afferent trigeminal nerve fibers and eventually leads to a reduction in breathing rate, inflammation, and, in the vomeronasal organ, prevents entrance of hazardous substances (Fig. 2d).

Similarly, bitter compounds exposure in the trachea caused vagal nerve activation and reduced breathing rate in an acetylcholine-dependent manner [27] (Fig. 2e). This was linked to tracheal tuft cells since they were shown to express the bitter taste signaling cascade components and acetylcholine related enzymes. Tracheal brush cells were also shown to be connected to cholinergic nerve fibers, although evidence for direct synapses between brush cells and nerve endings in the lower respiratory tract are lacking [112].

The discovery that the irritants which activate this pathway in both the upper airways and the trachea include bacterial products such as quorum sensing molecules, suggested that tuft cells may play a role in innate immunity [111,113,114]. It was also reported that the activation of tuft cell-like SCCs bitter taste receptor in the human upper airways causes an increase in intracellular calcium, which propagates to surrounding cells through gap junctions, and causes secretion of antibacterial compounds that can efficiently eliminate bacteria [115] (Fig. 2d). This local response was not seen in mice [116]. In spite of tuft cells expression of NOS and NADP-linked G6PD, and although ciliated cells of the upper airways were shown to produce NO in response to bitter taste receptor activation by bacteria [117], NO is not involved in the local anti-bacterial response in SCCs, but rather beta-defensins.

The interaction between tuft cells and the nervous system is not limited to the upper airways and urinary tract described above. In fact, in almost all tuft cells-containing tissues except for the thymus, tuft cells were found in close proximity to neurons, and evidence for direct synapses is accumulating [20,40,51,118,119]. The role of such interactions remains largely unknown and is likely tissue-dependent. Auditory tube and intestinal tuft cells were found near CGRP+ nerve fibers [20,68], which in the gut were shown to participate in neuroimmune interactions, nociception, blood pressure homeostasis and stem cells proliferation, thus implying that tuft cells regulate these functions. Specifically, the cross talk between tuft cells and neurons is important both for intestinal tuft cells survival in organoids and for expansion of the organoids [103], further supporting the role of tuft cells in epithelial proliferation and tissue regeneration, and suggesting that this requires their interaction with the nervous system.

In the thymus, whose main role is the generation of mature T cells, epithelial cells mediate the development and selection processes of thymocytes, and thus regulate the formation of the T-cell repertoire. Therefore, it is tempting to assume that thymic tuft cells are also involved in this process.

Thymic tuft cells, like tuft cells in other tissues, express ChAT and therefore might be able to secrete acetylcholine [73]. They are the main source of this enzyme in the thymic stroma [28]. It has been previously shown that acetylcholine affects T cells development and immune function, and is important for normal thymic function [69,120,121]. Therefore, it is possible that tuft cells play a role in shaping the adaptive immune repertoire through secretion of acetylcholine, although this requires further investigation.

Additionally, since thymic tuft cells are located in the medulla, it was recently hypothesized that similarly to mTECs, they are involved in specific antigen presentation, and thus have a role in preventing autoimmunity [29]. As described above, while tuft cells themselves do not express Aire at the RNA level, both Miller et al. and us demonstrated that some thymic tuft cells might arise from Aire expressing cells [28,29]. Both papers also showed that thymic tuft cells express low levels of CD74 and MHCII, molecules necessary for antigen presentation, which are not expressed by gut tuft cells. Finally, in thymic transplantation experiments, Miller et al. showed that thymic tuft cells deficiency led to formation of antibodies against IL25, a tuft cells-expressed gene, in mice immunized with IL25. Taken together, these observations may indicate tuft cells involvement in tolerance induction.

Additional roles for thymic tuft cells, which are not directly related to shaping the T cells repertoire, were also explored. As described above, tuft cells in the gut were shown to activate a type 2 immune response to eliminate parasites. While the thymus is usually not directly exposed to such external cues, it is able to sense and responds to them, for example it is known that parasitic or bacterial infection causes reversible involution of the thymus [122]. Therefore, and since thymic tuft cells express IL25, their role in type 2 response regulation was investigated. Interestingly, both Miller et al. and us found evidence for tuft cells effect on type 2 immune response-related cells populations, but while Miller et al. demonstrated that tuft cells deficiency resulted in reduced NKT2 cells and $EOMES^{+}CD8^{+}$ cells in the thymus and spleen, we showed an increase in basal levels of thymus-resident type-2 innate lymphoid cells (ILC2) in tuft cells deficient mice, perhaps representing feedback loops or intestine-thymus crosstalk. Therefore, determining the involvement of thymic tuft cells in type 2 immune response activation will require mice models which specifically eliminate these cells without affecting tuft cells in other tissues. Additional roles for thymic tuft cells, which might be thymus-specific, will require further investigation (Fig. 2f).

While varied, these proposed functions of tuft cells might not be contradictory, and may actually be related; For example, succinate is not only a parasitic metabolite but is also secreted endogenously, and is known to be elevated during tissue stress and injury [123]. Additionally, tissue remodeling is a part of the type 2 immune response [123], and specifically it was shown that ILC2 activation and IL13 secretion in the gut over a long period of time causes remodeling and lengthening of the intestine [99].

Furthermore, the currently proposed functions may be just the “tip of the iceberg”, since there are many characteristic tuft cells components, whose involvement in tuft cells activity is currently unknown and likely have a functional significance. This includes secretion of opioids [52,124], expression of renin and angiotensin [51] (likely related to water balance homeostasis), NO synthesis [54], etc. Tuft cells were also shown to be localized near CGRP+ nerve fibers, known to be involved in various functions including tissue remodeling, neuroimmune interactions, muscle contraction, maintenance of blood pressure homeostasis and more [68]. This can suggest tuft cells involvement in any of these processes. It is also possible, and even likely, that tuft cells function varies between different organs or regions, between states of health and disease, and between organisms.

Contributors

All authors contributed equally in writing this review.

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