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Taste Bud Homeostasis in Health, Disease, and Aging

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Abstract

The mammalian taste bud is an onion-shaped epithelial structure with 50–100 tightly packed cells, including taste receptor cells, supporting cells, and basal cells. Taste receptor cells detect nutrients and toxins in the oral cavity and transmit the sensory information to gustatory nerve endings in the buds. Supporting cells may play a role in the clearance of excess neurotransmitters after their release from taste receptor cells. Basal cells are precursor cells that differentiate into mature taste cells. Similar to other epithelial cells, taste cells turn over continuously, with an average life span of about 8–12 days. To maintain structural homeostasis in taste buds, new cells are generated to replace dying cells. Several recent studies using genetic lineage tracing methods have identified populations of progenitor/stem cells for taste buds, although contributions of these progenitor/stem cell populations to taste bud homeostasis have yet to be fully determined. Some regulatory factors of taste cell differentiation and degeneration have been identified, but our understanding of these aspects of taste bud homoeostasis remains limited. Many patients with various diseases develop taste disorders, including taste loss and taste distortion. Decline in taste function also occurs during aging. Recent studies suggest that disruption or alteration of taste bud homeostasis may contribute to taste dysfunction associated with disease and aging.

Key words: aging, cell death, cell renewal, disease, taste buds, taste dysfunction

Introduction

Taste buds are peripheral structures responsible for sensing taste compounds in food and drink. Each taste bud contains a number of specialized epithelial cells, including taste receptor cells for recognizing sweet, bitter, umami, sour, and salty compounds (Chandrashekar et al. 2006). It has been known for decades that taste cells turn over continuously throughout life. The average turnover rate of taste cells was reported to be about 8–12 days, although some cells in taste buds can survive much longer (Beidler and Smallman 1965; Farbman 1980; Hamamichi et al. 2006; Cohn et al. 2010; Perea-Martinez et al. 2013). The normal function of taste buds depends on a continuous supply of properly differentiated taste receptor cells. Disruption of taste tissue homeostasis can be detrimental to the taste system. Certain diseases and conditions, as well as normal aging can be associated with taste disorders (Bartoshuk 1989; Cowart 1989; Stevens 1996; Mojet et al. 2001; Heft and Robinson 2010). Taste abnormalities not only decrease quality of life but also contribute to anorexia, weight loss, and malnutrition. Development of taste disorders may vary depending on the underlying causes. Likewise, the presence of taste abnormalities in any given disease can be idiosyncratic. Recent studies suggest that aberrations in taste bud homeostasis, such as abnormal or suboptimal cell renewal, differentiation, and degeneration, are likely contributors to taste dysfunction associated with diseases, therapies, and aging (Wang et al. 2007; Cohn et al. 2010; Kim et al. 2012; Nguyen et al. 2012; Shin et al. 2012). In this review, we focus on the current knowledge on taste tissue homeostasis, its regulatory mechanisms, and how disease and aging may alter the balance of taste cell renewal and degeneration.

Taste bud cell types and molecular markers

Taste buds are distributed on the surface of the tongue, soft palate, and other oropharyngeal locations (Miller 1995). On the mammalian tongue, taste buds reside in fungiform, foliate, and circumvallate papillae. Caution should be exercised when using a novel animal model because these 3 tissue structures may not always be present (Kobayashi et al. 2005). Cells of the taste bud are derived from epithelial lineages but are specialized to perform gustatory functions. Taste receptor cells acquire certain characteristics associated with neurons, such as the ability to generate action potentials and to release neurotransmitters upon binding of appropriate ligands. Traditionally, classification of

taste bud cells was based on ultrastructural and morphological observations. The accepted generalized classification scheme of taste cells being divided into types I–IV (discussed below in this section) was solidified by Murray's ultrastructural observations on rabbit taste buds (Murray 1971). Recent studies have provided a number of molecular and immunohistological markers for these cell types (Finger 2005; Chaudhari and Roper 2010). In Table 1, we list some common cell-type markers for taste bud cells and taste progenitor/stem cells.

Type I cells are spindle-shaped cells with several long microvilli. Most type I cells are considered to have supportive or glia-like functions in taste buds, for instance, to hydrolyze the neurotransmitter adenosine 5′-triphosphate (ATP) after its release (Bartel et al. 2006). A subset of type I cells may also contribute to salty taste reception (Vandenbeuch et al. 2008). Type I cells can be identified by their expression of nucleoside triphosphate diphosphohydrolase-2 (NTPDase2), the human blood group antigen H, and the glial glutamate/aspartate transporter (GLAST) (Bartel et al. 2006). However, identification of type I cells using antibodies to these marker proteins can be problematic at times due to low levels of staining (for GLAST) or plasma membrane staining of cells that may wrap around other types of cells in taste buds (for NTPDase2).

Type II cells are polarized cells with multiple short microvilli. Type II cells are receptor cells for sweet, bitter, and umami tastes, and they release ATP as a neurotransmitter when activated by taste compounds (Finger et al. 2005). ATP release is likely through protein channels (Dando and Roper 2009; Kinnamon 2013; Taruno et al. 2013). Various signaling molecules involved in sweet, bitter, and umami taste transduction have been used as type II cell markers. Phospholipase C-β2 (PLC-β2) and transient receptor potential channel M5 (TrpM5), both required for sweet, umami, and bitter taste signaling, are expressed in most, if not all, type II taste cells (Perez et al. 2002; Zhang et al. 2003). The type 3 inositol 1,4,5-trisphosphate receptor (IP3R3) is another marker broadly expressed in type II cells and is involved in calcium release from intracellular storage sites upon activation of sweet, umami, and bitter taste receptors (Clapp et al. 2001; Miyoshi et al. 2001; Hisatsune et al. 2007). In contrast, the G-protein α subunit gustducin, another commonly used marker, is expressed in only a subset of type II taste cells. Additionally, the relative frequency of sweet, umami, or bitter cells within gustducin-expressing type II cells is different among taste fields (e.g., fungiform vs. circumvallate papillae) (Kim et al. 2003; Stone et al. 2007). The G-protein γ subunit 13 (Gγ13) is specifically expressed in type II cells in taste buds and is a component of the heterotrimeric G-protein complexes for sweet, umami, and bitter signaling (Huang et al. 1999). The expression and function of Gγ13 has also been noted in other sensory systems, such as vision and olfaction (Huang et al. 2003; Li et al. 2013). In addition, messenger RNA in situ hybridization for type 1 (sweet and umami) and type 2 (bitter) taste receptors (T1Rs and T2Rs) is commonly used to reveal subsets of type II cells (Hoon et al. 1999; Adler et al. 2000; Matsunami et al. 2000; Max et al. 2001).

Type III cells are thin spindle-shaped cells with single microvillus. Type III cells can form synapses with gustatory nerve endings (Chaudhari and Roper 2010). Subsets of type III cells are involved in sour and salty taste reception (Huang et al. 2008; Kataoka et al. 2008; Oka et al. 2013). In addition, type III cells may play a role in integrating and transmitting signals from neighboring type II cells to gustatory nerves (Tomchik et al. 2007). Type III cell markers include neural cell adhesion molecule (NCAM), synaptosomal-associated protein 25 (SNAP25), PKD2L1 (a polycystic-kidney-disease-like ion channel), carbonic anhydrase 4 (Car4), aromatic l-amino acid decarboxylase (AADC), and serotonin (Nelson and Finger 1993; Yang et al. 2000; Yee et al. 2001; DeFazio et al. 2006; Huang et al. 2006; Kataoka et al. 2008; Chandrashekar et al. 2009). Some of these markers, such as NCAM and SNAP25, are expressed not only in type III cells but also in some nerve fibers that course among the taste bud cells.

Type IV cells, or basal cells, are round-shaped cells that reside near the base of taste buds (Finger 2005). These cells are newly generated taste precursor cells that differentiate into mature taste cells. The best known basal cell marker is sonic hedgehog (Shh), and in situ hybridization using Shh antisense probes labels basal cells (Miura et al. 2001; Hall et al. 2003). Hairy and enhancer of split 6 (Hes6) and prospero-related homeobox 1 (Prox1) transcription factors have also been found in basal cells (Seta et al. 2006).

Table 1 Molecular markers for taste progenitor/stem cells and taste bud cells

Cell type	Marker
Progenitor/stem cell	K14, K5, p63, Sox2, Ki67, Lgr5
Basal cell	Shh, Hes6
Type I cell	GLAST, NTPDase2, antigen H
Type II cell	PLC-β2, TrpM5, IP3R3, gustducin, Gγ13, T1Rs, T2Rs
Type III cell	NCAM, SNAP25, PKD2L1, Car4, AADC, serotonin

Ki67, antigen identified by monoclonal antibody Ki 67; PKD2L1, polycystic kidney disease 2-like 1; T1R, taste receptor type 1; T2R, taste receptor type 2.

Taste cell turnover and taste bud homeostasis

Pulse-chase labeling experiments and taste cell turnover

Taste buds undergo continuous cellular renewal, differentiation, and degeneration (Figure 1A). The survival period of taste cells, from their birth to degeneration, can be estimated using DNA pulse-chase labeling methods. Early studies several decades ago used tritium-labeled thymidine (Beidler and Smallman 1965; Conger and Wells 1969), whereas more recent studies utilized 5-bromo-2′-deoxyuridine (BrdU) or 5-ethynil-2′-deoxyuridine (EdU) to label newly synthesized DNA (Hamamichi et al. 2006; Cohn et al. 2010; Perea-Martinez et al. 2013). Using these methods, the average life span of taste cells in adult taste buds was estimated to be 8–12 days, but subsets of taste cells can survive for a much long period of time (Hamamichi et al. 2006;

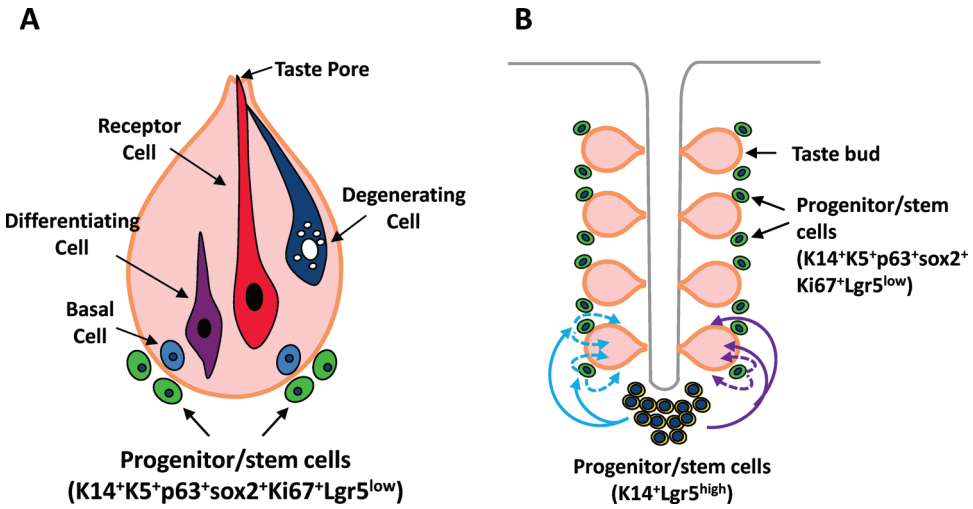

Figure 1 Schematic drawing of taste bud turnover and renewal. (**A**) The process of taste bud cell turnover. Cells at different stages of renewal and turnover are labeled. The progenitor/stem cell population ($K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$) in the basal region outside of the taste bud is illustrated. (**B**) Taste bud renewal. In circumvallate and foliate papillae, 2 populations of taste progenitor/stem cells have been identified: one at the epithelial base outside of taste buds ($K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$) and the other at the bottom of the trench ($K14^+Lgr5^{high}$). Two scenarios of taste bud renewal are proposed. In the first scenario (left, blue arrows), $K14^+Lgr5^{high}$ cells give rise to $K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$ cells, which in turn engender taste bud and perigemmal cells. In the second scenario (right, purple arrows), $K14^+Lgr5^{high}$ cells and $K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$ cells both give rise to taste bud and perigemmal cells through independent lineages.

Cohn et al. 2010; Perea-Martinez et al. 2013). In addition, different types of taste cells may follow distinct time courses for differentiation and degeneration (Delay et al. 1986; Cho et al. 1998; Perea-Martinez et al. 2013). A recent study showed that type II cells labeled by a PLC-β2 antibody had a half-life of 8 days, whereas the half-life of serotonin-positive type III cells was 22 days (Perea-Martinez et al. 2013).

Except for basal cells, which are located in the basal region of taste buds, differentiating, mature, and dying taste cells cannot be reliably identified based on their location in taste buds. In fact, it remains enigmatic where and how aged taste cells are removed from the taste buds during normal taste cell turnover. In a BrdU pulse-chase labeling experiment, we followed the position of BrdU-labeled nuclei in taste buds at different time points after BrdU injection. Double immunostaining was carried out using antibodies to BrdU and KCNQ1, a voltage-gated potassium channel protein that is a marker of all taste cells in mouse (Wang et al. 2009a). Positions of BrdU-labeled nuclei in taste buds were recognized based on immunostaining images and recorded on taste bud maps (Figure 2). BrdU-labeled nuclei outside of taste buds were not considered. These maps confirmed that 1 day after BrdU injection, the majority of labeled nuclei from newborn taste cells were located in the basal region of taste buds. Five days after BrdU injection, labeled nuclei spread all over taste buds. At later time points, especially 30 days after BrdU injection, there was a tendency for BrdU-labeled nuclei to reside in the upper portion and close to the edge of taste buds, suggesting that some aged taste cells might be leaving the taste buds through the upper edge during routine cell turnover (Figure 2, lower right panel).

Taste bud progenitor and stem cells

Pulse-chase labeling experiments also revealed that the majority of labeled cells shortly after a pulse of ^{3}H-thymidine (Beidler and Smallman 1965) or BrdU (Hamamichi et al. 2006) are located in the basal region outside of the taste buds. These labeled cells represent dividing or newborn cells, and their localization suggests that most, if not all, proliferating cells in the taste epithelium reside outside of the taste buds. This conclusion is also supported by data from immunohistochemical studies using antibodies to cell proliferation markers, such as Ki67 and proliferating cell nuclear antigen. Cells that are positive for these proliferation markers are almost exclusively located outside of taste buds and mostly in the basal region close to taste buds (Hamamichi et al. 2006; Cohn et al. 2010; Nguyen and Barlow 2010). These proliferating cells likely represent dividing taste progenitor/stem cells that give rise to progenies, some of which enter taste buds, becoming basal cells (Figure 1A). A recent study reported the observation of a small percentage of Ki67-positive cells inside taste buds (Sullivan et al. 2010). However, it remains unclear whether these cells have the ability to self-renew or to give rise to other types of taste cells.

Recently, mouse genetic models for cell lineage mapping have been used to study taste progenitor/stem cells. Thirumangalathu et al. (2009) used a *Shh*creER mouse line that expressed an inducible Cre recombinase (creER) under the control of a Shh promoter to study the contribution of Shh-expressing embryonic taste placodes to taste buds in fungiform papillae. The embryonic Shh-expressing progenitors gave rise to type I and II taste cells,

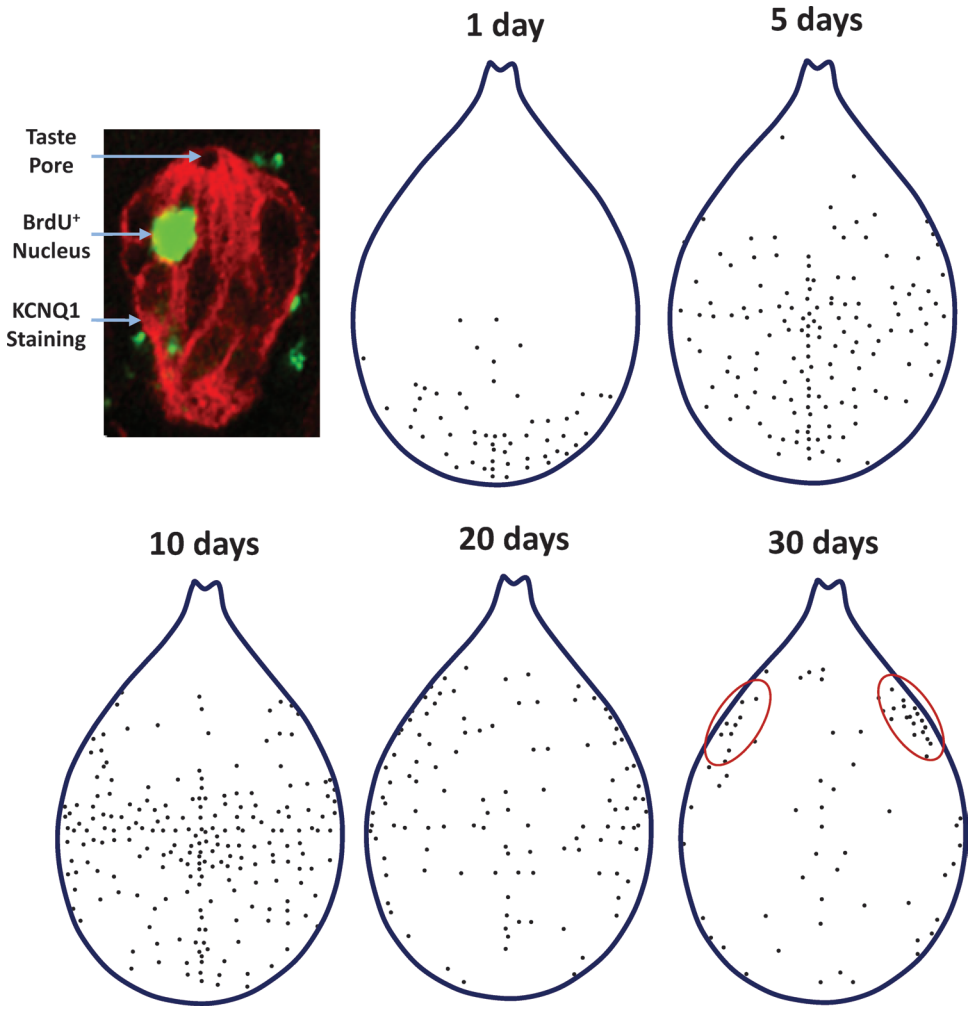

Figure 2 Maps of BrdU-labeled nuclei from a pulse-chase experiment. Taste tissues were collected at the indicated time points after BrdU injection. Circumvallate tissue sections were processed for double immunostaining using antibodies against BrdU and KCNQ1. Fluorescence images were taken using a confocal microscope. A representative overlay image of a taste bud is shown in the upper left panel, with BrdU staining in green and KCNQ1 staining in red. The boundaries of taste buds were identified based on KCNQ1 immunostaining. BrdU-positive nuclei inside taste buds were identified and their positions were recorded on taste bud maps. About 50 taste buds were included for each post injection time point. One day after BrdU injection, most of BrdU-labeled nuclei were located at the basal region of taste buds. Five and 10 days after injection, BrdU-labeled nuclei were distributed all over taste buds. Thirty days post injection, many of the remaining BrdU-labeled nuclei were located close to the upper limit of the taste buds (red circles).

as well as intragemmal basal and perigemmal edge cells in newborn and young adult mice. However, the embryonic Shh-expressing progenitors and their descendants did not include long-term adult taste progenitor/stem cells because the lineage-labeled cells disappeared from taste buds when mice reached 4 months of age (Thirumangalathu et al. 2009). Using a transgenic line with a keratin 14 (K14) promoter–controlled creER (K14-creER), Okubo et al. (2009) studied $K14^+$ cells and their progeny in neonatal (postnatal day 2) and young adult (postnatal day 30) mice. This study showed that $K14^+$ progenitor/stem cells can give rise to taste bud cells in fungiform papillae, circumvallate papillae, and soft palate, as well as keratinocytes surrounding taste buds and taste pores, suggesting that $K14^+$ progenitor/stem cells are bipotential (i.e., they give rise to both taste cells and perigemmal keratinocytes). Immunostaining using antibodies to K14 and other stem cell markers showed that $K14^+$ taste bud progenitor/stem cells are localized to the basal region outside of taste buds. These cells are also positive for keratin 5 (K5), transformation related protein 63 (Trp63), and SRY (Sex-Determining Region Y)-Box 2 (Sox2) (Okubo et al. 2009).

Two recent studies suggest that the leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) is another, perhaps more specific, progenitor/stem cell marker for taste buds (Takeda et al. 2013; Yee et al. 2013). Using Lgr5-GFP mice, Yee et al. (2013) detected strong expression of Lgr5 in a population of cells at the bottom of each circumvallate and foliate trench and weak expression at the epithelial base outside of circumvallate and foliate taste buds. $Lgr5^+$ cells appear to be a subset of $K14^+$ cells in circumvallate and foliate papillae. Genetic tracing experiments were conducted

in adult mice using *Lgr5-EGFP-IRES-creERT2* and *Rosa26-LacZ* double-transgenic line, as well as *Lgr5-EGFP-IRES-creERT2* and *Rosa26-tdTomato* double-transgenic line. Both transgenic lines can be induced by tamoxifen to genetically label cell lineages derived from $Lgr5^+$ cells. The results showed that $Lgr5^+$ cells can give rise to perigemmal epithelial cells and types I, II, and III taste bud cells in circumvallate and foliate papillae (Yee et al. 2013). This study identified a new niche for taste progenitor/stem cells at the bottom of circumvallate and foliate trench where $K14^+Lgr5^{high}$ cells reside (Figure 1B). In addition, it is in agreement with the report by Okubo et al. (2009), suggesting that perigemmal epithelial cells and taste bud cells are derived from same populations of progenitor/stem cells. Takeda et al. (2013) also reported Lgr5 expression in the basal regions outside of circumvallate taste buds. Both neonatal and adult mice express Lgr5 in circumvallate papillae. Furthermore, Lgr5 is expressed in fungiform papillae from neonatal mice. However, its expression declines and becomes undetectable in fungiform papillae of adult mice. Lineage tracing experiments also suggest that adult taste progenitor/stem cells are Lgr5-negative in fungiform papillae but are Lgr5-positive in circumvallate papillae (Takeda et al. 2013).

These studies suggest that the progenitor/stem cells for fungiform taste buds of neonatal and young mice are different from those of older mice. In neonatal and young mice, these progenitor/stem cells are derives from Shh^+ taste placode cells during embryonic development, which gradually disappear from adult fungiform papillae. Instead, another population of progenitor/stem cells, coming from a different embryonic lineage, becomes the major source for cell renewal of adult fungiform taste buds. The identity and embryonic lineage of these adult progenitor/stem cells remain unclear.

In circumvallate and foliate papillae, there may also be 2 populations of taste progenitor/stem cells for taste buds: one at the epithelial base of taste buds and the other at the bottom of circumvallate and foliate trench (Figure 1B). The former population is known to be $K14^+$, $K5^+$, $p63^+$, $sox2^+$, and $Lgr5^{low}$, whereas the latter is $K14^+$ and $Lgr5^{high}$. It remains unclear how the 2 populations of progenitor/stem cells relate to each other and what their respective contributions are to circumvallate and foliate taste buds. In one scenario, $K14^+Lgr5^{high}$ cells at the bottom of trenches give rise to $K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$ cells at the base of taste buds, which in turn give rise to perigemmal cells and taste bud cells (Figure 1B, left, blue arrows). In this scenario, $K14^+Lgr5^{high}$ cells represent taste stem cells, whereas $K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$ cells represent transient amplifying taste progenitor cells. In another scenario, $K14^+Lgr5^{high}$ cells and $K14^+K5^+p63^+sox2^+Ki67^+Lgr5^{low}$ cells are 2 independent populations of taste progenitor/stem cells that can both give rise to perigemmal and taste bud cells through unrelated lineages (Figure 1B, right, purple arrows). More experiments are needed to distinguish these possibilities.

Regulatory factors of taste lineage specification and taste cell differentiation

Multiple morphogenetic factors have been shown to regulate the number, size, and patterning of taste papillae during embryonic development, including Wnt, Shh, bone morphogenetic proteins, epidermal growth factor, and fibroblast growth factors (Hall et al. 2003; Liu et al. 2004; Zhou et al. 2006; Iwatsuki et al. 2007; Liu et al. 2007, 2008; Beites et al. 2009; Petersen et al. 2011). In contrast, the factors that regulate adult taste cell lineage specification are less clear. Sox2, a transcription factor, was shown to be critical for fungiform papilla and taste bud formation during development (Okubo et al. 2006). Sox2 is expressed in some taste bud cells, as well as taste progenitor/stem cells located in the basal region outside of taste buds (Suzuki 2008; Okubo et al. 2009). It has been suggested that Sox2 may regulate the differentiation of taste bud cells versus keratinocytes (Okubo et al. 2006). Shh and Notch signaling pathways are the other candidates for regulating taste cell fate determination. Shh is expressed in embryonic taste placodes, as well as in basal cells of adult taste buds (Miura et al. 2001; Hall et al. 2003). As mentioned above, Shh-expressing embryonic taste progenitors give rise to at least 2 types (type I and II) of taste cells in young animals (Thirumangalathu et al. 2009). In adult taste buds, the expression of Shh and its receptor, Patched1 (Ptc), depends on gustatory nerve innervation of taste buds. Denervation causes rapid loss of expression of both genes, preceding the disappearance of taste buds (Miura et al. 2004). The reappearance of Shh and Ptc expression correlates with the formation of new taste buds, suggesting that Shh and Ptc may play a role in taste cell lineage determination.

Many components of the Notch signaling pathways are expressed in developing and adult taste tissues, including Notch receptors, their ligands, and downstream transcription factors (Seta et al. 2003). Hes1, Hes6, and Mash1 are basic helix–loop–helix transcription factors involved in Notch signaling and in neural lineage determination and differentiation. Hes6 is expressed in a subset of basal cells (Seta et al. 2003, 2006). Its function in taste buds remains unclear. Hes1 is also expressed in a subset of taste bud cells (Seta et al. 2003). A recent study suggests that Hes1 may play a role in suppressing the expression of PLC-β2 and IP3R3 in non–type II taste cells. In Hes1-deficient embryos (embryonic day 18) or newborn mice (postnatal day 0), the number of PLC-β2- and/or IP3R3-positive cells in circumvallate papillae was more than 5-fold greater than in wild-type littermates (Ota et al. 2009). However, whether this effect of Hes1 persisted into adulthood was not examined, as Hes1-deficient mice died at birth. Mash1 was observed in round basal cells and a subset of differentiated type III cells (Seta et al. 2006). In Mash1-knockout mice, the expression of AADC, a type III cell marker, was not observed in the developing circumvallate and soft palate taste buds from

embryonic day 18.5 embryos (Seta et al. 2011). However, the number and size of taste buds in the soft palate of Mash1-knockout embryos were comparable with those of wild-type embryos. The expression of some other type III cell markers, including SNAP25 and NCAM, was still detected in the soft palate epithelium of Mash1-knockout mice. This study suggests that Mash1 may be required for the differentiation of the AADC-positive subset of type III cells or for the expression of some type III cell-specific genes (Seta et al. 2011).

The homeodomain transcription factor Skn-1a was shown to be crucial for generating sweet, umami, and bitter taste receptor cells (Matsumoto et al. 2011). Skn-1a is expressed in a small number of basal taste cells, as well as type II taste cells. Skn-1a-knockout mice lack the expression of T1Rs, T2Rs, gustducin, PLC-β2, and TrpM5 in taste buds and do not respond to sweet, umami, and bitter compounds. In the taste buds of Skn-1a-knockout mice, there is an expansion of type III cells shown by the increased number of cells expressing PKD2L1, PKD1L3, AADC, and SNAP25 (Matsumoto et al. 2011).

Mechanisms for taste cell death

On average, 11% of taste cells are replaced daily in the adult mouse taste buds (Zeng et al. 2000). It has been suggested that apoptosis is a major mechanism for cell death in taste buds (Zeng and Oakley 1999). At any given time, in adult taste buds, the regulators for apoptosis, such as p53, Bax, and caspase-2, could be detected in 8–11% of cells. p53 and caspase-2 frequently colocalize with Bax in double-immunostaining experiments. In contrast, in postnatal day 7 mice, when most taste cells were young, no more than 1% of taste cells were positive for either p53 or Bax (Zeng and Oakley 1999). Furthermore, in BrdU dating experiments, Bax immunoreactivity was detected only in older taste cells (at least 5 days old), suggesting that Bax-dependent apoptosis occurs in aged cells. Taste buds of *Bax* knockout mice were reported to contain more than twice the normal number of taste cells, and circumvallate papillae from these mice are longer and taller (Zeng et al. 2000). These studies indicate that aged taste cells may use Bax- and caspase-dependent pathways to commit cell death.

Because recent studies suggest that different types of taste cells have distinct life spans, it is possible that divergent mechanisms are used for initiating and/or committing cell death in taste buds. Ueda et al. (2008) used an anti-single-stranded DNA (anti-ssDNA) antibody to detect DNA breaks as an early indication for apoptosis (Ueda et al. 2008). In rat circumvallate papillae, immunohistochemical staining showed that about 78% of ssDNA-positive cells were also positive for the human blood group antigen H that labels mostly type I cells. About 16.8% and 14% of ssDNA-positive nuclei were immunoreactive to antibodies against PLC-β2 and gustducin (type II cell markers), respectively. However, no ssDNA-positive cells were found immunoreactive to an antibody against NCAM, a type III cell marker. If the ssDNA antibody reliably labels apoptotic cells in taste buds, these results suggest that more type I cells at any given time are undergoing apoptosis than other types of taste cells. In addition, type III cells may use different pathways for cell death, as no ssDNA-positive type III cells were detected. It remains to be seen whether the specificity of the method can be further confirmed.

Some cell cycle regulators may be involved in the control of cell death in taste buds. The cyclin-dependent kinase inhibitor $p27^{Kip1}$ is expressed abundantly in mature taste bud cells but not in basal cells (Hirota et al. 2001). In mice deficient for $p27^{Kip1}$, apoptosis was increased in taste buds as shown by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay and histology (Harrison et al. 2011), suggesting that $p27^{Kip1}$ may play a role in suppressing cell death in taste buds.

In addition, cell death can be induced by various pathological triggers, such as denervation, diseases, toxins, chemotherapy, and radiation therapy. Cell death under these conditions may employ additional pathways for initiation and execution.

Taste dysfunction in disease and aging

Taste dysfunction associated with diseases

Upper respiratory viral infections and oral cavity infections are among the most common causes of taste dysfunction (Henkin et al. 1975; Bartoshuk et al. 1987; Goodspeed et al. 1987; Cullen and Leopold 1999; Bromley 2000). Bartoshuk et al. (1987) reported that upper respiratory infections often accompanied localized taste losses on the tongue. Hypogeusia and dysgeusia develop frequently in patients following an influenza-like illness (Henkin et al. 1975). AIDS, caused by HIV infection, is also frequently associated with taste disorders (Graham et al. 1995; Heald and Schiffman 1997; Heald et al. 1998). Heald et al. (1998) reported that up to 67% of AIDS patients surveyed complained of taste alterations. The detection thresholds of glutamic acid and quinine hydrochloride were significantly higher in HIV-infected patients (Graham et al. 1995). Increased detection and recognition thresholds for various taste stimuli have also been reported in patients with viral hepatitis (Smith et al. 1976).

Several autoimmune diseases are known to affect taste function (Campbell et al. 1983; Bromley and Doty 2003). For example, Sjögren's syndrome patients display decreased taste acuity to bitter, sour, salty, and sweet compounds (Henkin et al. 1972; Weiffenbach et al. 1995; Gomez et al. 2004). Sjögren's syndrome is an autoimmune disorder characterized by the infiltration of immune cells into salivary glands and several other tissues (Fox 2005; Jonsson et al. 2007). Although saliva production is affected in the majority of these patients, the amount of saliva produced does not seem to directly correlate with taste impairment (Weiffenbach et al. 1995; Gomez et al. 2004; Reiter et al.

2006). Taste dysfunction also occurs in systemic lupus erythematosis (SLE), another autoimmune disorder that can affect several organs and tissues (Campbell et al. 1983; Bromley and Doty 2003). Several other autoimmune and/or inflammatory diseases, such as inflammatory bowel diseases, rheumatic arthritis, and diabetes, can also affect taste (Campbell et al. 1983; Gondivkar et al. 2009; Steinbach et al. 2011).

Many cancer patients experience taste alterations. Decreased sensitivity to sweet and salty compounds may develop in some patients (Carson and Gormican 1977), whereas increased sensitivity to bitter compounds may also occur (DeWys and Walters 1975). Taste abnormalities in patients correlate with reduced energy intake and poor prognosis (DeWys 1977). The rapid growth of cancer and its associated tissue damage and inflammation may contribute to this aberration of taste. In addition, cancer treatments, such as chemotherapy and radiation therapy, can further exacerbate the problem (Sherry 2002). Up to two-thirds of the patients receiving chemotherapy report some degree of taste dysfunction, which may begin during the course of treatment and last for several months after treatment (Wickham et al. 1999). Head and neck cancer patients receiving radiation therapy also frequently report taste dysfunction, including decreased abilities to detect and recognize taste compounds (Mossman et al. 1982; Mirza et al. 2008).

Damage to the peripheral and/or central nervous system may affect taste. It is well known that injury of the chorda tympani nerve during ear surgery can result in taste disturbances (Saito et al. 2001; Michael and Raut 2007). Some patients with neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, show taste impairment, although taste alterations in these patients are generally less severe than olfactory dysfunction (Shah et al. 2009; Steinbach et al. 2010; Kashihara et al. 2011).

Aging and taste loss

The effects of aging on the sense of taste have been the subject of investigation for decades. Many studies have reported a general decline in taste sensitivity in elderly subjects (Bartoshuk 1989; Cowart 1989; Stevens 1996; Mojet et al. 2001; Heft and Robinson 2010). Aging is associated with increased thresholds for taste detection and recognition (Cowart 1989; Stevens 1996; Mojet et al. 2001). Intensity ratings of taste compounds also decline with age (Cowart 1989; Schiffman 1997). The effects of aging on different taste qualities may vary. Although some controversies exist, most studies show that sensitivities to salty and bitter tastes show more substantial decreases than do sensitivities to sour and sweet tastes (Cowart 1989; Murphy and Gilmore 1989; Frank et al. 1992; Stevens 1996). Yet, despite their common occurrence in elderly populations, taste alterations are often unnoticed or ignored.

Taste bud homeostasis in disease and aging

Taste bud homeostasis under disease conditions

Homeostasis of taste buds is generally well maintained throughout the life span of organisms. However, disturbances of structural balance of taste buds can occur under various pathological conditions. Abnormal taste buds and tongue papillae have been observed in Sjögren's syndrome patients with or without oral candidiasis (Henkin et al. 1972; Hernandez and Daniels 1989; Yamamoto et al. 2009). Henkin et al. (1972) reported decreased number of fungiform papillae and taste buds in Sjögren's syndrome patients. Taste buds from patients' circumvallate papillae were also reduced in number and showed histological characteristics of cellular degeneration when examined by electron microscopy (Henkin et al. 1972).

We also observed structural defects in taste tissues from MRL/MpJ-Fas^{lpr}/J (MRL/lpr) mice, a model for Sjögren's syndrome and systemic lupus. MRL/lpr mice carry a mutation in the *Fas* gene that abolishes the expression and function of *Fas* and results in autoantibody production and development of systemic autoimmunity at several months of age (Watanabe-Fukunaga et al. 1992). The autoimmune disease in MRL/lpr mice resembles human SLE and Sjögren's syndrome. Taste tissues of diseased MRL/lpr mice contain infiltrated T lymphocytes and express higher levels of several cytokines, including tumor necrosis factor (TNF) and interferon-γ (IFN-γ), suggesting increased local inflammatory responses. Taste buds from these mice are smaller than those from wild-type control mice. Reverse transcription–polymerase chain reaction (RT-PCR) and immunohistochemical analyses all suggest that the number of type II taste cells is reduced in MRL/lpr mice. Gustatory nerve recordings and behavioral tests show reduced responses to bitter and sweet compounds. To investigate whether taste cell renewal was affected in these mice, we performed BrdU pulse-chase experiments. The results showed that fewer BrdU-positive newborn cells entered taste buds of MRL/lpr mice compared with control mice, suggesting that taste cell renewal is inhibited in this mouse model of autoimmune disease (Kim et al. 2012).

Taste cell renewal is also inhibited in the lipopolysaccharide (LPS)-induced inflammation model (Cohn et al. 2010). LPS treatment in mice rapidly induces the expression of several inflammatory cytokines in the taste epithelium. BrdU pulse-chase experiments showed that LPS significantly reduces the number of BrdU-labeled cells in the basal region outside of taste buds 1 day after LPS and BrdU injections, suggesting an inhibition of proliferation of taste progenitor/stem cells at this niche. RT-PCR analyses revealed that LPS treatment markedly reduced the expression of Ki67, cyclin B2, and E2F1 in the taste epithelium. The latter 2 are important regulators of cell cycle progression, whereas Ki67 is a cell proliferation marker. Immunostaining using antibodies to Ki67 confirmed that the number of Ki67-positive cells was significantly decreased in the basal regions outside of taste buds by LPS. Accordingly,

fewer BrdU-labeled newborn cells entered the taste buds after LPS treatment, suggesting that taste cell renewal is inhibited by LPS. In addition, LPS moderately shortened the average life span of taste cells, indicating increased cell death in taste buds, which may be induced by inflammatory cytokines such as IFN-γ (Wang et al. 2007).

Activation of inflammatory pathways may alter taste bud homeostasis and contribute to the development of taste disorders associated with various diseases. Inflammatory factors can be induced in many acute and chronic illnesses, such as infection, autoimmunity, and cancer. Compared with nontaste lingual epithelium, taste buds express higher levels of multiple genes involved in inflammatory responses (Wang et al. 2007; Hevezi et al. 2009; Wang et al. 2009b; Feng et al. 2012; Shi et al. 2012). Some cytokines are expressed preferentially in subsets of taste bud cells. We have shown that the inflammatory cytokine TNF is predominantly produced by T1R3-positive sweet and umami receptor cells in mice (Feng et al. 2012). IFN-γ is produced by SNAP-25-positive type III cells and a subset of PLC-β2-positive type II cells (Kim et al. 2012). The anti-inflammatory cytokine IL-10 is selectively expressed by gustducin-positive bitter receptor cells in mouse circumvallate and foliate taste buds (Feng P, Chai J, Zhou M, Simon N, Huang L, Wang H, unpublished data). Figure 3 summarizes the expression of TNF, IFN-γ, IL-10, and their receptors in different types of taste bud cells. The significance of this cell type–specific expression of cytokines is unclear. In clinical situations, some patients develop taste loss of a specific modality (Huque et al. 2009). It is possible that a particular combination of cytokines produced under certain disease conditions contributes to this modality-specific taste loss. Furthermore, many inflammation-associated cytokines are multifunctional regulators of cell proliferation, differentiation, and cell death. These cytokines may mediate the effect of inflammation on taste progenitor/stem cell proliferation, taste cell lineage specification, taste cell differentiation, and cell death (Figure 4).

Head and neck irradiation and chemotherapy drugs can also disrupt taste bud homeostasis (Figure 4). Irradiation in cancer patients and in animal models induces loss of taste cells and taste buds (Conger and Wells 1969; Zain and Hanks 1989; Nelson 1998; Yamazaki et al. 2010; Nguyen et al. 2012). Conger and Wells (1969) showed that localized head irradiation rapidly decreased the number of taste buds and number of cells per bud. The numbers dropped to minimum at about 9 days after irradiation and recovered by about day 20. A later study reported that a single 15-Gy dose of X-ray irradiation preferentially reduced the number of gustducin-positive type II cells compared with the number of serotonin-positive type III cells (Yamazaki et al. 2010). It is possible that this preferential effect is related to the faster turnover rate of type II cells compared with type III cells (Perea-Martinez et al. 2013). Irradiation also diminishes the number of taste progenitors located at the basal region outside of taste buds (Yamazaki et al. 2010; Nguyen et al. 2012). These taste progenitor cells undergo cell cycle arrest or apoptosis within 1–3 days after irradiation but resume proliferation at 5–7 days (Nguyen et al. 2012). Interruption of taste cell renewal may be the primary cause of taste loss after irradiation. Chemotherapy drugs, such as cyclophosphamide, have also been shown to disrupt the homeostasis of taste buds and papillae (Mukherjee and Delay 2011; Mukherjee et al. 2013).

The maintenance of taste bud structure in adult taste tissues is highly dependent on the gustatory nerves. Transection of gustatory nerve fibers causes extensive taste cell degeneration by apoptosis (Guth 1957; Fujimoto and Murray 1970; Takeda et al. 1996; Huang and Lu 2001; Guagliardo and Hill 2007). The expression of Shh and its receptor Ptc diminishes rapidly after denervation (Miura et al. 2004). These observations suggest that taste bud innervation is essential not only for the survival of taste cells but also for taste lineage specification (Figure 4). During ear surgery, damage to the chorda tympani nerve, which innervates fungiform papillae and the anterior parts of foliate papillae, often leads to loss of taste buds and disturbance of taste function (Michael and Raut 2007; Saito et al. 2011).

Taste bud homeostasis in aging

Most studies in humans and animals have reported structural declines in the aging taste tissue. Reported changes include reduced total number of taste buds, reduced taste bud density in the epithelium, and reduced number of taste cells per taste bud. Back in the 1930s, a comprehensive study by Arey et al. (1935) showed that the average number of taste buds in human circumvallate papillae decreased substantially in the 74- to 85-year-old age group (74–85 years) (Arey et al. 1935), an observation supported by several later studies (Mochizuki 1937, 1939; Shimizu 1997). Related to this attribute, the density of taste buds in the epithelium also decreased in the elderly (Shimizu 1997; Kano et al. 2007). In animal studies, Conger and Wells (1969) showed that the number of taste buds in mouse circumvallate papillae, as well as the number of cells in each taste bud, decreased with age (Conger and Wells 1969). A recent study by Shin et al. (2012) also reported a significant reduction in circumvallate taste bud size and the number of taste cells per bud in 18-month-old mice compared with 2- and 10-month-old controls (Shin et al. 2012).

The mechanisms that cause age-associated declines in the peripheral taste structures remain mostly unclear. It has been reported that the immunoreactivity of Shh in taste buds is significantly reduced in aged mice and rats compared with young adult controls (Shin et al. 2012; Cai et al. 2013), suggesting that taste cell lineage specification may be affected. Shin et al. (2012) also reported that the percentage of cells expressing T1R3 and some taste modulators was decreased in aged mice. In addition, it is generally believed that the number and/or proliferative potential of adult tissue progenitor/stem cells decrease with age (Jang et al. 2011; Jones and Rando 2011). To investigate whether aging affects

Cell Type	Type I	Type II		Type III
	Salty?	Sweet/ Umami	Bitter	Sour
TNF	-	+	-	-
TNFR	+	+	+	+
IFN-γ	nd	+*		+
IFNGR	nd	+*		+
IL-10	-	-	+	-
IL-10R	-	+	-	-

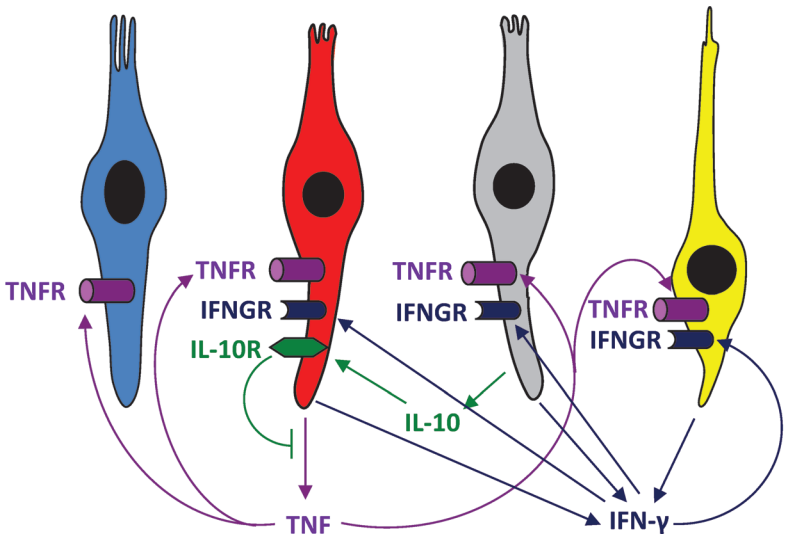

Figure 3 Expression of TNF, IFN-γ, IL-10, and their receptors in different types of taste bud cells. In mouse circumvallate and foliate taste buds, TNF is predominantly produced by T1R3-positive sweet/umami receptor cells. TNF receptors (TNFR) are expressed in most taste bud cells. IFN-γ and its receptors (IFNGR) are selectively expressed by a subset of type II cells and most type III cells. The anti-inflammatory cytokine IL-10 is selectively produced by bitter receptor cells in mouse circumvallate and foliate taste buds, whereas IL-10 receptors (IL-10R) are preferentially expressed in T1R3-positive sweet/umami cells (Feng P, Chai J, Zhou M, Simon N, Huang L, Wang H, unpublished data). IL-10 is known to inhibit the production of inflammatory cytokines, such as TNF. The expression of IL-10R in TNF-producing cells (sweet/umami cells) suggests that IL-10 signaling may downregulate TNF production in taste buds. nd, not determined; +*, expression was detected in a subset of PLC-β2-positive cells, but specific expression in sweet/umami and bitter cells has not been determined.

taste progenitor/stem cells, we examined Ki67$^+$ cells in the basal regions outside of circumvallate taste buds. Double immunostaining was carried out using antibodies against Ki67 and KCNQ1. We then quantified the number of Ki67$^+$ cells associated with each taste bud using images from the immunostaining. As shown in Figure 5, we found a significant reduction in the number of Ki67$^+$ cells in circumvallate epithelium from aged mice (18–20 months old) compared with young adult mice (4–6 months old). This result indicates that aging decreases the number of proliferating taste progenitor/stem cells, which may reduce the ability for taste buds to regenerate after various damaging events, such as inflammation, chemical toxicity (e.g., chemotherapy drugs), or nerve damage. A study by He et al. (2012) showed that, after chorda tympani nerve sectioning, the functional regeneration of the peripheral taste structure is markedly delayed in aged rats (He et al. 2012). However, it remains unclear whether this delay is due to reduced capacities in gustatory nerve fiber regeneration, taste bud regeneration, or both. Together, aging may affect taste bud homeostasis at several levels, including reduction in the number of taste progenitor/stem cells, suboptimal regulation of taste lineage specification, and decline in the expression of critical regulators of taste cell differentiation (Figure 4).

Conclusion

Taste bud homeostasis is maintained through balanced cell renewal, differentiation, and cell death. Recent research has made important advances concerning taste progenitor and stem cells, although many other aspects of taste cell renewal remain to be elucidated. A few regulatory factors

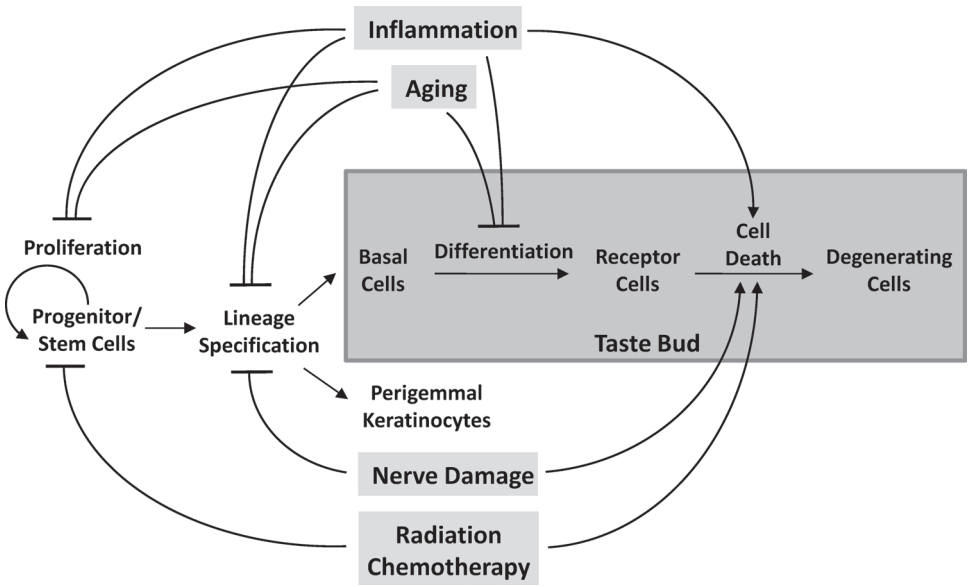

Figure 4 Effects of inflammation, aging, gustatory nerve damage, radiation, and chemotherapy on taste bud homeostasis. Inflammation induced by various diseases, such as infection and autoimmunity, can inhibit proliferation of taste progenitor/stem cells, specification of newborn cells into gustatory cell lineages, and differentiation of basal cells. Inflammation can also induce cell death of taste receptor cells. Aging may affect taste bud homeostasis through reduction of taste progenitor/stem cell populations and suboptimal regulation of taste lineage specification and taste cell differentiation. Gustatory nerve damage can induce degeneration of taste receptor cells and may deprive the signal for taste lineage specification. Radiation and chemotherapy induce apoptosis of taste receptor cells and inhibit taste progenitor/stem cell proliferation. Disrupting the normal pace of cell renewal and death in taste buds may lead to taste dysfunction.

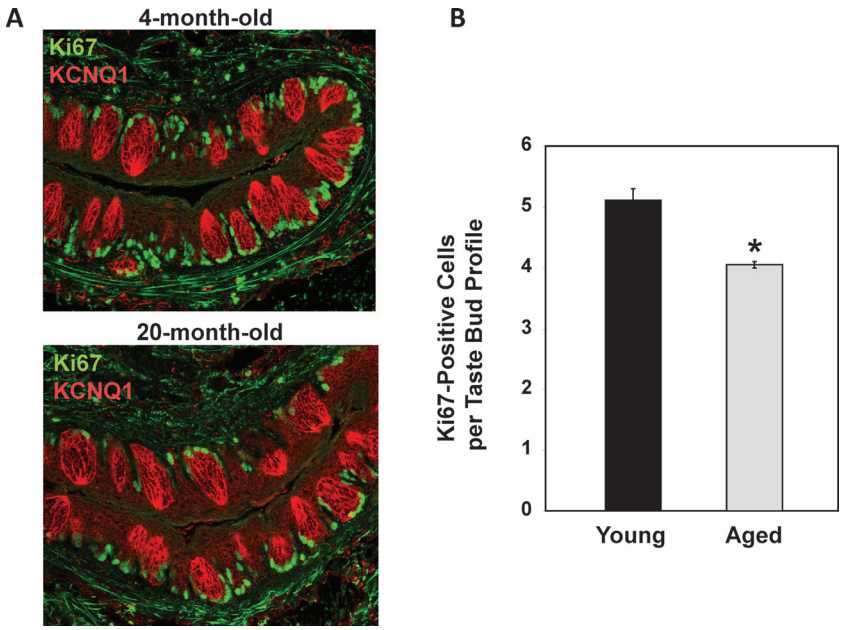

Figure 5 Reduced number of proliferating progenitor/stem cells in the basal regions outside of circumvallate taste buds of aged mice. Taste tissue sections were processed for immunostaining with antibodies against Ki67 (green) and KCNQ1 (red). (**A**) Confocal images of circumvallate papillae from a young (4-month-old) mouse and an aged (20-month-old) mouse. (**B**) Reduced number of Ki67$^+$ cells in circumvallate epithelium from aged mice (18–20 months old) compared with young adult mice (4–6 months old). *$P < 0.05$. $N = 5$.

of taste cell differentiation have been recognized recently. However, the regulatory mechanisms for taste cell degeneration during normal turnover remain largely unknown. Taste bud homeostasis can be disrupted under various disease conditions, by gustatory nerve damage, or by exposure to toxic compounds. Inflammation, as a common factor in many diseases, may contribute significantly to taste dysfunction by deregulating taste cell renewal, differentiation,

and degeneration. Furthermore, a substantial amount of evidence suggests that the sense of taste declines with age. Diminished taste function in the elderly is accompanied by reduced numbers of taste buds and taste cells. The number of proliferating taste progenitor/stem cells also seems to decline in old age, at least in rodents. This may compromise the capacity of peripheral taste tissue to regenerate after exposure to various insults. Further understanding of taste tissue homeostasis may lead to the development of treatment strategies for taste disorders.

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