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REVIEW

A new fate for old cells: brush cells and related elements

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Abstract

Over the past 50 years, hundreds of studies have described those cells that are characterized by a brush of rigid apical microvilli with long rootlets, and which are found in the digestive and respiratory apparatuses. These cells have been given names such as brush cells, tuft cells, fibrillovesicular cells, multivesicular cells and caveolated cells. More recently, it has been realized that all these elements may represent a single cell type, probably with a chemosensory role, even if other functions (e.g. secretory or absorptive) seem to be possible. Very recent developments have permitted a partial definition of the chemical code characterizing these elements, revealing the presence of molecules involved in chemoreceptorial cell signalling. A molecular cascade, similar to those characterizing the gustatory epithelium, seems to be present in these elements. These new data suggest that these elements can be considered solitary chemosensory cells with the presence of the apical 'brush' as an inconsistent feature. They seem to comprise a diffuse chemosensory system that covers large areas (probably the whole digestive and respiratory apparatuses) with analogies to chemosensory systems described in aquatic vertebrates.

Key words brush cell; gustducin; solitary chemosensory cell; taste; tuft cell.

The concept of brush cells

'A rose is a rose, but what is a brush cell?' asked Thurlbeck (1990) in a comment on a paper describing brush cells (BCs) in human fetuses. Fourteen years later, the question seems to have an answer. The history of the BCs starts about 45 years ago, with the introduction of electron microscopy, when several 'new' cell types were described on the basis of typical ultrastructural features. One of these was the BC. The first description of BCs is generally attributed to Rhodin & Dalham (1956) in the rat trachea. Since this first description, the presence of BCs has been confirmed in the airway of several species, including humans (Rhodin, 1959). Eight years after their discovery, it was realized that BCs are also present in the lung (Meyrick & Reid, 1968) and in the digestive apparatus (Luciano et al. 1968a,b). In particular, it was discovered that BCs are particularly concentrated in the gallbladder (Luciano & Reale, 1969).

For several years, the description of this enigmatic cytotype was not substantially modified. The identification of BCs was morphological and mainly based on the presence of a brush of apical microvilli with long rootlets. However, isolated cells with the ultrastructural features of BCs had previously been identified in several organs and indicated with different names, so that a systematic review of the literature appears to be difficult. Isolated non-endocrine cells with the typical apical end have been indicated as brush, tuft, caveolated or fibrillo-vesicular cells, or with other names, and reviews of these cells in different apparatuses do not exist. In addition, recent data suggest that the concept of BCs must be changed, and that ultrastructure together with neurochemistry and molecular biology could provide a more adequate definition of these elements. In particular, the recent description of gustducin (Hofer & Drenckhahn, 1998) and other bitter taste-related molecules in these elements localized in the digestive and respiratory apparatuses demonstrated a link between these cells and elements of the taste buds. These results strongly supported the idea that BCs may operate as

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solitary chemoreceptors, and show the need for a more detailed analysis of what the BCs described in the last five decades really are.

Synonyms

In the respiratory epithelia, all authors use the term BCs (or type III pneumocytes as synonymous of alveolar BCs) and these cells are largely assumed to be receptors.

In the various epithelia of the gastrointestinal tract, the cells were given different names (e.g. fibrillovesicular, multivesicular, caveolated, tuft cells) and secretory or absorptive functions were proposed. Luciano & Reale (1997) noted that this irregularity in nomenclature did not contribute to our knowledge of BCs; rather it served to spread the erroneous idea that BCs in the gastrointestinal and respiratory tracts represent different cell types. Although morphological variation suggests that all these cells may belong to a single family, minor differences in the ultrastructure of the cells in the various locations are described. Therefore, in the present review we report on the peculiarity of the subfamilies described during the last 45 years of study.

Tuft cells

The term 'tuft cell' is the one most commonly used in the literature, together with 'brush cell'. The discovery of tuft cells is generally attributed to Jarvi & Keyrilainen (1955), who described an unusual epithelial cell in the stomach characterized by an apical tuft of long microvilli. The term indicates the subfamily of BCs located in the digestive apparatus (Sato et al. 2000) but it is generally not used by authors studying the respiratory apparatus.

Caveolated cells

The discovery of caveolated cells in the gastrointestinal system is generally attributed to Nabeyama & Leblond (1974). The term refers to the caveolae present in the apical cytoplasm of the cells. The term is considered synonymous with tuft cells, to indicate a subfamily of BCs of the digestive apparatus and in particular of the gastric mucosa.

Fibrillovesicular cells

The BCs located at the distal wall of the groove between the rat forestomach and glandular stomach were called fibrillovesicular cells (Hammond & LaDeur, 1968). Further studies (Wattel et al. 1977a,b; Wattel & Geuze 1978) demonstrated that the fibrillovesicular cells are characterized by long microvilli, apical bundles of microfilaments and a complex 'tubulovesicular system', like BCs located in other districts.

Other names

In addition to those reported above, Sato & Miyoshi (1997) cited a series of other terms used to indicate BCs: multivesicular cells (Silva, 1966), undifferentiated cells (Johnson & Young, 1968), s-cells (Ferguson, 1969) or agranular light cells (Riches, 1972). We have not found use of these terms in the recent literature.

The BCs of the respiratory apparatus

Airways

After their description by Rhodin & Dalhamn (1956) in the rat trachea, the presence of BCs was confirmed in the rabbit (Leeson, 1961). In the following years, several studies provided a detailed description of airway BCs in different mammalian species (Rhodin, 1966; Luciano et al. 1968a,b; Jeffery & Reid, 1975; Ishida, 1977; Taira & Shibasaki, 1978; Christensen et al. 1987).

In general, BCs represent a population of epithelial cells scattered throughout the epithelial lining of the respiratory system. These cells are reliably distinguished from other epithelial cells only at the ultrastructural level, by the presence of an apical tuft of stiff microvilli and extremely long microvillar rootlets that may project down to the perinuclear space. BCs can be identified in tissue sections even at the light microscopic level by immunostaining with antibodies against villin and fimbrin, two proteins that crosslink actin filaments to form bundles (Hofer & Drenckhahn, 1992). Not only are villin and fimbrin in BCs present in the actin filament core bundles of apical microvilli and their long rootlets but, in addition, both proteins are also associated with microvilli extending from the basolateral cell surface of BCs.

Nasal cavity

In the respiratory epithelium of the rat nasal mucosa, BCs were studied by Monteiro-Riviere & Popp (1984). These cells are pear-shaped, with the broad base adjacent to the basement membrane and large microvilli on

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the surface. Microfilaments, microtubules, vesicles and paired cisternae were found in the apical cytoplasm. BCs occurred singly on the conchae and lateral wall but were not identified in the respiratory epithelium of the nasal septum.

Alveolar epithelium of the lung

After their description in the airways, BCs were also found in the alveolar epithelium of the lung (Meyrick & Reid, 1968). Several studies confirmed this report (Luciano et al. 1969; Hijiya et al. 1977; Allan, 1978; Filippenko, 1978; Hijiya, 1978; Foliguet & Grignon, 1980; Chang et al. 1986). In rats, alveolar BCs, also called type III pneumocytes, are a normal component of alveolar lining cells. Based on different studies, they have an incidence ranging from 5–10% to 0.5–1% of the pneumocyte population (Meyrick & Reid, 1968; Foliguet & Grignon, 1980). The highest density is at the bifurcation of the first alveolar duct (Chang et al. 1986). Alveolar BCs lack the lamellated inclusion of type II pneumocytes. The BCs are largely covered by type I pneumocytes. The cell apex shows straight microvilli of variable length. Each microvillus contains fine filaments that extend into the cytoplasm but do not appear to integrate into a terminal web. Beneath this elaborate microvillus border there are vesicles and aggregates of glycogen. Desmosomes are rarely seen except near the lumen as part of the junctional complex.

The BCs of the rat lung can be identified at the light and electron microscope level by antibodies against both cytokeratin 18 and the actin-crosslinking protein villin (Kasper et al. 1994). At the ultrastructural level, microvilli and their rootlets in the apical cytoplasm were labelled by antivillin antibodies, whereas a monoclonal antibody against cytokeratin 18 labelled bundles of intermediate filaments.

It has been suggested that lung BCs are chemoreceptors. An interesting hypothesis suggests that they are active in regulating capillary resistance and perfusion during hypoxia, acting by contracting structures located in the interstitial space (Hijiya et al. 1977; Hijiya, 1978). It has also been suggested that BCs are involved in regenerative processes (Filippenko, 1981).

Vomeronasal organ epithelium

BCs are also localized in the nasal cavity and, in particular, the non-sensory epithelium of the rat vomeronasal organ contains BCs (Hofer et al. 2000).

Human respiratory apparatus

Several studies also confirmed the presence of BCs in the human respiratory apparatus (Rhodin, 1959). Watson & Brinkman (1964) studied the trachebronchial tree, Rhodin (1966) the trachea and Basset et al. (1971) the bronchioles.

The brush/tuft/caveolated/fibrillovesicular/multivesicular cells of the digestive apparatus

Tuft cells in the salivary ducts

Cells with strong analogies to BCs are the tuft cells of the salivary ducts, which are characterized by long microvilli with prominent rootlets and by vesicular and tubular profiles. Sato & Miyoshi (1996, 1997) and Sato et al. (2000) studied the tuft cells in the main excretory duct epithelia of rat salivary glands. These cells exhibited similar fundamental characteristics in the salivary glands and in other organs. Numerous membrane-bound electron-dense granules were present among the microvilli of the tuft cells in the submandibular gland, but not in other organs. The apical cytoplasm contained numerous vesicles with a filamentous substance that reacts positively for glycoconjugates. The vesicles were frequently close to the apical plasma membrane and seemed to open into the lumen. Nerve endings with synaptic vesicles were seen close to the basal portion of the tuft cells. The functions of the tuft cells in the salivary glands were suggested to be secretion, absorption and reception.

Stomach

BCs were found in the epithelium of the fundic region mucosa of the hind stomach in the *Llama guanacoe* (Luciano et al. 1980) and of rodents. In the rat stomach, BCs are mainly localized in a restricted region, the limiting ridge separating the forestomach from the glandular stomach (Luciano & Reale, 1992). In rats, the forestomach is separated from the glandular stomach by a fold of the forestomach mucosa, which generates the 'limiting ridge' on the inner surface of the organ. This ridge overlaps a deep groove, which is flanked proximally by the forestomach and distally by the glandular stomach. Light microscopy and scanning electron microscopy reveal that the keratinized squamous

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epithelium of the forestomach merges into the columnar epithelium of the glandular stomach at the bottom of the groove. Among the columnar cells of the distal wall of the groove are numerous BCs. The peculiar architecture of the 'limiting ridge' and the presence of numerous BCs in its distal wall suggest that the region not only represents the transitional zone between forestomach and glandular stomach but that it might have a more specific function. Luciano et al. (1993) demonstrated that isolated BCs of the rat stomach retain their structural polarity after isolation and considered this finding to be further confirmation of their receptorial differentiation.

Kugler et al. (1994) showed that BCs (also called caveolated cells) of the rat gastric cardia and major pancreatic duct display strong immunoreactivity for nitric oxide synthase (NOS) and also exhibit high activity of NADPH-diaphorase. This NADPH-oxidizing activity was previously shown to be mediated by a specific domain of the sequence of the NOS. NADPH, in turn, appears to be delivered by glucose-6-phosphate dehydrogenase, which is expressed in BCs at particularly high levels. The authors concluded that BCs of the stomach and pancreas may represent a specialized population of paracrine cells that use nitric oxide as a messenger molecule to control certain gastrointestinal functions.

Recently, Luciano et al. (2003) demonstrated that BCs of rodent gallbladder and stomach epithelia express neurofilaments. The results demonstrated that the BCs of both organs express two types of intermediate filaments, i.e. neurofilaments and cytokeratin 18 filaments, and that these have a compartmentalized distribution in the cytoplasm. BCs did not express peripherin. The immunodetection of intermediate filaments distinctive for mature neurons in BCs supported their putative receptor function.

Lymphoid follicle-associated epithelium in the gastric mucosa

Lymphoid follicle-associated epithelium in the gastric mucosa displayed BCs interposed between mucous epithelial cells. These BCs were characterized by prominent microfilament bundles and many apical vesicles or caveola and specifically enveloped the clusters of intraepithelially invading lymphocytes and macrophage-like cells through thin cytoplasmic sheets similar to M cells in Peyer's patches (Wada et al. 2000).

Gallbladder

The BCs of the gallbladder have been studied in a series of works by Luciano & Reale (1969, 1979, 1990) and Luciano et al. (1981). Their results were summarized in a review Luciano & Reale (1997). These works demonstrated that BCs are the second most frequent cellular component of the epithelium of the mouse gallbladder and that they are present in large numbers toward the neck and in the fundic regions of the organ. In the gallbladder, BCs have a narrow apical portion, bulky body and basal cytoplasmic projections. Their microvilli form a prominent brush border and have more similarity with stereocilia of sensory cells than with conventional microvilli. Freeze-fracture replicas demonstrate that, like stereocilia, the P face of the microvilli plasma membrane of BCs is smoother than the E face, but several intramembranous particles form small aggregates on the microvillus tip of both P and E faces. In the apical cytoplasm, the cytoskeleton consists of bundles of actin filaments originating from the axis of the apical microvilli and stretching continuously up to the supranuclear region of the cell. Microtubules flank the actin filaments in an alternating manner. Owing to the strong parallel arrangement of both cytoskeletal structures, the apical cytoplasm of the BCs assumes a typical stiffness. Vesicles of different sizes are aligned between the bundles of actin filaments and microtubules. Intraluminal injection of horseradish peroxidase demonstrates that these vesicles are not resorptive as they are not filled by the tracer. The BCs possess a large number of lateral microvilli appearing as rigid cytoplasmic protrusions. The bundle of actin filaments emanating from each lateral microvillus extends at different angles into the cytoplasm. A conspicuous number of bundles of 10-nm filaments are intertwined around the nucleus and extend toward the desmosomes of the lateral plasma membrane and into the basal cellular body. The authors reported several arguments in support of the view that interactions between the plasma membrane with its differentiations, on the one hand, and the cytoskeleton elements, on the other, play a key role in the function of the BC as a receptor (sensory) cell.

Common bile duct

The BCs of the common bile duct of the rat were studied by Luciano et al. (1981). Numerous BCs were seen in the proximal and distal regions of the common bile duct. In

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these locations, they could be implicated in a registration and/or regulation of intraluminal pressure variations. Two different fixative procedures (immersion and perfusion) and four different fixative solutions were used in order to obtain the best preservation of the BCs of the common bile duct of the rat. The results indicated that only perfusion fixation through the common bile duct is suitable, independent of the fixative solutions and their osmolarity.

In a more recent study, Iseki (1991) showed that the BCs in the common bile duct of the rat continue to develop postnatally and after 16 weeks they comprise about 30% of the whole population. He also showed that about one-quarter of the BCs are immunoreactive for liver fatty acid binding protein (L-FABP).

Small and large bowel

Intestinal BCs were first described in the rat (Luciano et al. 1968a,b). These cells have been the object of several studies and represent a model for understanding the structure of the whole family of BCs. At the light microscopic level, intestinal BCs can be identified by antibodies against the actin filament crosslinking proteins villin and fimbrin, which not only stain the apical tuft of microvilli and their rootlets, but also label projections emanating from the basolateral surface of these cells (Hofer & Drenckhahn, 1996). BCs can be distinguished at the neighbouring simple epithelium of the stomach, pancreatic duct and duodenum by particularly strong immunoreactivity with antibodies specific for cytokeratin 18. Tubulin antibodies reacted strongly with the upper half of BCs in a pattern not observed in the other epithelial cells of these tissues, including enteroendocrine cells of the duodenum (Hofer & Drenckhahn, 1996). Ankyrin, a protein that links the spectrin-based membrane cytoskeleton to integral proteins of the plasma membrane was revealed as a third cytoskeleton-associated protein, prominently expressed in BCs where ankyrin is restricted to the basolateral membrane domain (Hofer & Drenckhahn, 1996). The apparently high concentration of cytokeratin 18, tubulin and ankyrin in BCs suggests that these cytoskeletal proteins might play a role in the mechanical stability and polarized organization of these putative receptor cells.

The glycocalyx of intestinal BCs was studied by Gebhard & Gebert (1999). In this study the glycocalyx contained significantly higher amounts of L-fucose residues as detected by the lectins UEA-I and LTA than did enterocytes. In contrast, most of the other lectins bound more avidly to the glycocalyx of enterocytes. The cytoplasmic vesicles closely resembled the apical membrane in their labelling pattern. Quantification of the distribution of BCs revealed that the epithelia of the Peyer's patches contained ten-fold higher numbers of BCs than the small intestinal mucosa distant from lymphoid tissue. The authors concluded that BCs possess a glycocalyx with a specialized composition and differ significantly from enterocytes. Because similar peculiarities of the apical membrane have previously been described for sensory cells of the olfactory and gustatory organs, this study provides further evidence in favour of a sensory function of BCs.

In a further study, Gebert et al. (2000) demonstrated that the apical membrane of intestinal BCs possesses a specialized, but species-specific, composition of glycoconjugates by use of on-section and *in vivo* lectin labelling in rats, guinea-pigs and mice. Lectin binding sites were consistently located in the glycocalyx of the apical membrane and in that of cytoplasmic vesicles. *In vivo* lectin labelling revealed that the glycoconjugates of the apical membrane are accessible under physiological conditions, that BCs do not endocytose and that they probably possess a high membrane turnover rate. The results showed that specializations exist in the composition of glycoconjugates forming the glycocalyx of BCs in all species investigated. The presence of BC-specific glycoconjugates would be in accordance with the current hypothesis of a receptive function of BCs. Differences in the specific glycosylation patterns among rats, guinea-pigs and mice indicate that species-specific adaptations exist.

In the mucous membrane of the large intestine of the ox, sheep and goat, BCs were found in the epithelium and were characterized by electron-lucid spheroids (45×35 nm in diameter), in the supranuclearly located osmiophilic granules (Wille, 2001).

Pancreas

The major pancreatic excretory ducts have been shown to contain a large number of BCs. These are usually studied together with gastric and intestinal BCs. Information on these cells has been provided by Kugler et al. (1994), who showed that the BCs of the major pancreatic duct display strong immunoreactivity for NOS and also exhibit high activity of NADPH-diaphorase.

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In addition, pancreatic BCs have been described by Hofer & Drenckhahn (1996, 1998) in studies that showed that BCs are concentrated in the terminal portions of extralobular ducts and in the major pancreatic duct, where they comprise up to 22% of the ductal epithelium.

BCs in amphibians and fishes

The term BC has been mainly used for mammals. In other vertebrate species, similar elements are usually given different names such as solitary chemosensory cells or oligovillous cells. However, Whitear (1992) noted that the BCs of the bile duct (Luciano et al. 1981) and tracheal epithelium (Luciano et al. 1968a,b) of the rat have a fine structure strikingly similar to that of the chemosensory cells in fish, especially lampreys, with apical microvilli and microtubules and vesicles in the subapex. The term BC was used to describe elements located in the gastric mucosa of tadpoles (Sugimoto et al. 1985).

In the intestine of catfish *Corydoras aeneus* the thin-walled posterior intestine is adapted to air breathing. In this region, the mucosa is lined with respiratory epithelium and solitary BCs with several long and thick microvilli present (Podkowa et al. 2002).

BCs in the human fetus

DiMaio et al. (1990) performed a morphological examination of human fetal lung tissue, using scanning and transmission electron microscopy. BCs characterized by a border of regular straight microvilli containing a filamentous core were observed within the tracheal epithelium of a fetus of 19–20 weeks gestational age. These cells constituted 0.5% of the total epithelial cell population. BCs were not seen within the bronchial, bronchiolar or developing acinar epithelium. The study showed that BCs occur infrequently but normally in the developing tracheal epithelium of the second trimester fetus.

BCs in disease

Despite their widespread presence in several organs only a few works investigated the question of a possible involvement of BCs in pathological processes. In the past, some studies demonstrated an involvement of the BCs in diseases. In the respiratory apparatus, BCs have been claimed to be involved in bronchitis (Watson & Brinkman, 1964) and immotile cilia syndrome (Gordon & Kattan, 1984). A spontaneous pathology with involvement of BCs was also reported by DiMaio et al. (1988). They studied a full-term infant which developed bilateral pneumothoraces and respiratory distress shortly after birth. The biopsy revealed desquamative interstitial pneumonitis with the unique ultrastructural demonstration of numerous alveolar BCs.

The possible role of BCs in airway pathology was confirmed by studies on laboratory animals.

An experimental rat model used bleomycin to produce interstitial pneumonitis and a marked increase in alveolar BCs (Hijiya et al. 1977; Hijiya, 1978). Inflammatory thickening of the alveolar septum was followed by accumulation of macrophages and type II pneumocytes in the alveolar space. The prominence of the BCs seemed to be a regenerative response to bleomycin injury.

Increased numbers of BCs have been found in rat lung undergoing compensatory hypertrophy after unilateral pneumonectomy (Filippenko, 1981). It has also been reported that in rat trachea exposed to low-frequency noise the BCs fuse their microvilli (Branco et al. 2004).

In the literature, we have found only a single description of a spontaneous pathology with involvement of BCs in the digestive apparatus. A fibrillo-caveolated carcinoma has been described (Carstens et al. 1976) in which the tumour cells showed morphological similarities to the caveolar cells of the digestive apparatus.

However, some *in vitro* data seem to confirm the role of BCs in digestive neoplasm. The presence of 'tuft' (caveolated) cells in two human colon carcinoma cell lines (LIM 1863 and LIM 2211) was reported (Barkla et al. 1988). The presence of caveolated cells in LIM 1863 colon carcinoma cell line was then confirmed by a further study. In this cell line, characteristic morphological features of tuft cells included a wide base, narrow apex and a tuft of long microvilli projecting from the apical surface. The microvilli were attached by a core of long microfilaments passing deep into the apical cytoplasm. Between the microvilli were parallel arrays of vesicles (caveoli) containing flocculent material. Two different explanations for the presence of tuft cells were proposed. The first explanation is that tuft cells originated from the resected tumour and had survived by mitotic division during subsequent passages. The second explanation suggests that tuft cells are the progeny of undifferentiated tumour cells. The

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morphology of tuft cells was consistent with that of a highly differentiated cell specialized for absorption.

Gustducin in BCs

The alpha-subunit of the trimeric G-protein complex specific for taste receptor cells of the tongue, alpha-gustducin, was described in the stomach and intestine (Hofer et al. 1996). The alpha-gustducin-containing cells were identified as BCs that are scattered throughout the surface epithelium of the gut and share structural features of taste receptor cells of the tongue. These findings provided clues to the long-sought molecular and cellular basis for chemoreception in the gut. In a further study, Hofer & Drenckhahn (1998) provided further data on the identification of the taste cell G-protein, alpha-gustducin, in BCs of the rat pancreatic duct system. Because of some structural similarities to taste receptor cells of the tongue, the authors addressed the question of whether pancreatic BCs contain the taste cell-specific GTP-binding protein, alpha-gustducin, and hence might be considered to be involved in intraductal chemoreception. By immunostaining, the work showed that ductal BCs of the rat pancreatic duct system contain alpha-gustducin, which is concentrated in the apical tuft of microvilli and is also found along the basolateral cell surface. Immunoblotting of the major pancreatic duct revealed a 42-kDa band that co-migrated with alpha-gustducin of the rat tongue. Considering that ductal BCs are particularly rich in NOS-I, the authors assumed that these cells might play a role in certain aspects of chemoreceptive signalling and that chemosensory control of pancreatic secretion might occur at two independent sites, the intestine and the terminal portions of the excretory duct system.

In a review, Hofer et al. (1998) concluded that in the gut epithelium, scattered epithelial cells sharing apical cytoskeletal features of gustatory receptor cells were identified as BCs (tufted cells). These cells are rich in NOS and contain in their apical brush border the gustatory trimeric G-protein, alpha-gustducin, indicating that BCs are involved in chemoreceptive signalling.

BCs non-immunoreactive for α-gustducin

Despite the presence of gustducin in BCs of the digestive and respiratory apparatuses, it has been clearly demonstrated not all BCs display α-gustducin. BCs of the vomeronasal brush border do not contain α-gustducin (Hofer et al. 2000). Further studies appear to be necessary to assess if these elements contain G-proteins structurally different from gustducin.

BCs and solitary chemosensory cells

The solitary chemosensory cells (SCCs) are elements present in aquatic vertebrates (Kotrschal, 1991, 1996; Whitear, 1992). In fish, SCCs form a system of differentiated sensory epithelial cells, which are not organized into discrete end organs and may occur in the epithelia of oropharynx, gills and skin (Whitear, 1992). SCCs have recently been discovered in mammals (Sbarbati et al. 1998, 1999, 2004; Finger et al. 2003; Merigo et al. 2004) but they are not BCs (i.e. they do not possess the apical brush of rigid microvilli with long rootlets). However, α-gustducin-immunoreactive SCCs showing the ultrastructural features of BCs were found in the larynx (Sbarbati et al. 2004). These elements co-localize other molecules of the taste transductory cascade, including phospholipase C of the beta 2 subtype and IP_3R3 (Sbarbati et al. 2004). This finding suggests that a chemosensory role can also be maintained by BCs in the respiratory apparatus, probably in driving reflexes against airborne substances.

General considerations about BCs and related elements

Recent data confirm the hypothesis of Luciano & Reale (1969) that the BCs of the respiratory and digestive apparatus and the tuft/caveolated/fibrillovesicular cells of the digestive apparatus are the same cytotype, or at least belong to the same family, sharing a common phenotype with minor organ-related differences. The ultrastructural morphology of these cell types is quite similar and a large majority of authors today use the two terms as synonymous.

The extensive use of light and ultrastructural cytochemistry has allowed recent studies to acquire numerous data about these cells. In particular, it appears interesting that a large majority of them contain the G-protein gustducin, which is a strong marker of chemoreceptive cells. This strongly supports the hypothesis that these elements have a chemoreceptorial role, as suggested by several authors on the basis of morphological features. This does not mean that the other proposed roles (mechanoceptive, paracrine, exocrine, endocrine, absorptive, regenerative) are invalid, but

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rather that they could coexist in elements that have chemoreceptive capability. This is in accordance with the differentiated apical pole of the cell, which is its most characteristic ultrastructural feature. These cells represent true unicellular 'organs' and this explains their multiple faces. In general, they seem to configure a diffuse chemosensory system that covers large areas (if not the whole digestive and respiratory apparatuses), having analogies with the chemosensory systems described in aquatic vertebrates.

However, the data in the literature must be analysed with caution because in some cases a single cell type has been given different names whereas, in other cases, cells with different characteristics have been named BCs. In addition, the term 'brush cell' is also used for a population of neurons. Recent data demonstrate that the apical brush of microvilli must be considered an organelle (or better, a specialization of the apical membrane) and it is evident that to classify the cells on the basis of the presence of a single organelle can be misleading, because it can be present in different cell types. To date, a better way to identify a cell type seems to be a chemical coding of the most characteristic molecules that it contains. Using this approach, morphological data are also more easily correlated with a specific function. The chemical coding of BCs has provided important information, demonstrating the presence of molecules involved in chemoreception. These new data suggest that the term SCCs is more correct for these elements, as the presence of the apical 'brush' is not a constant feature. Therefore, the apical brush of microvilli seems to be a differentiation present in a subfamily of SCCs probably localized in specific microenvironments.

The richness of cytoplasmic vesicles that is visible in some of these elements seems also to suggest a secretory role. In these cells, the coexistence of chemoreceptive and secretory mechanisms could allow secretory reflexes in response to modifications of the microenvironment.

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References

Allan EM (1978) The ultrastructure of the brush cell in bovine lung. *Res. Vet. Sci*. **25**, 314–317.

Barkla DH, Whitehead RH, Foster H, Tutton PJ (1988) Tuft (caveolated) cells in two human colon carcinoma cell lines. *Am. J. Pathol*. **132**, 521–525.

Basset F, Poirier J, Le Crom M, Turiaf J (1971) Ultrastructural study of the human bronchiolar epithelium. *Z. Zellforsch Mikrosk. Anat*. **116**, 425–442.

Branco N, Santos J, Monteiro E, Silva A, Ferreira J, Pereira M (2004) The lung parenchyma in low frequency noise exposed Wistar rats. *Rev. Port Pneumolfeb* **10**, 77–85.

Carstens PH, Broghamer WL Jr, Hire D (1976) Malignant fibrillo-caveolated cell carcinoma of the human intestinal tract. *Hum. Pathol*. **7**, 505–517.

Chang LY, Mercer RR, Crapo JD (1986) Differential distribution of brush cells in the rat lung. *Anat. Rec*. **216**, 49–54.

Christensen TG, Breuer R, Hornstra LJ, Lucey EC, Snider GL (1987) The ultrastructure of hamster bronchial epithelium. *Exp. Lung Res*. **13**, 253–277.

DiMaio MF, Dische R, Gordon RE, Kattan M (1988) Alveolar brush cells in an infant with desquamative interstitial pneumonitis. *Pediatr. Pulmonol*. **4**, 185–191.

DiMaio MF, Kattan M, Ciurea D, Gil J, Dische R (1990) Brush cells in the human fetal trachea. *Pediatr. Pulmonol*. **8**, 40–44.

Ferguson DJ (1969) Structure of antral gastric mucosa. *Surgery* **65**, 280–291.

Filippenko LN (1978) Light and electron microscopic study of rat lung brush alveolocytes. *Biull. Eksp. Biol. Med*. **86**, 592–596.

Filippenko LN (1981) Possibility of formation of 'brush' cells from Type II alveolocytes in rats. *Biull. Eksp. Biol. Med*. **92**, 616–620.

Finger TE, Bottger B, Hansen A, Anderson KT, Alimohammadi H, Silver WL (2003) Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. *Proc. Natl Acad. Sci. USA* **100**, 8981–1986.

Foliguet B, Grignon G (1980) Type III pneumocyte. The alveolar brush-border cell in rat lung. Study by transmission electron microscopy. *Poumon. Coeur*. **36**, 149–153.

Gebert A, al-Samir K, Werner K, Fassbender S, Gebhard A (2000) The apical membrane of intestinal brush cells possesses a specialised, but species-specific, composition of glycoconjugates – on-section and in vivo lectin labelling in rats, guinea-pigs and mice. *Histochem. Cell Biol*. **113**, 389–399.

Gebhard A, Gebert A (1999) Brush cells of the mouse intestine possess a specialized glycocalyx as revealed by quantitative lectin histochemistry. Further evidence for a sensory function. *J. Histochem. Cytochem*. **47**, 799–808.

Gordon RE, Kattan M (1984) Absence of cilia and basal bodies with predominance of brush cells in the respiratory mucosa from a patient with immotile cilia syndrome. *Ultrastruct. Pathol*. **6**, 45–49.

Hammond JB, LaDeur L (1968) Fibrillovesicular cells in the fundic glands of the canine stomach: evidence for a new cell type. *Anat. Rec*. **161**, 393–411.

Hijiya K, Okada Y, Tankawa H (1977) Ultrastructural study of the alveolar brush cell. *J. Electron Microsc. (Tokyo)* **26**, 321–329.

Hijiya K (1978) Electron microscope study of the alveolar brush cell. *J. Electron Microsc. (Tokyo)* **27**, 223–227.

Hofer D, Drenckhahn D (1992) Identification of brush cells in the alimentary and respiratory system by antibodies to villin and fimbrin. *Histochemistry* **98**, 237–242.

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Hofer D, Drenckhahn D (1996) Cytoskeletal markers allowing discrimination between brush cells and other epithelial cells of the gut including enteroendocrine cells. *Histochem. Cell Biol*. **105**, 405–412.

Hofer D, Puschel B, Drenckhan D, (1996) Taste receptor-like cells in the rat gut identified by expression of α-gustducin. *Proc. Natl Acad. Sci. USA* **93**, 6631–6634.

Hofer D, Drenckhahn D (1998) Identification of the taste cell G-protein, alpha-gustducin, in brush cells of the rat pancreatic duct system. *Histochem. Cell Biol*. **110**, 303–309.

Hofer D, Jons T, Kraemer J, Drenckhahn D (1998) From cytoskeleton to polarity and chemoreception in the gut epithelium. *Ann. NY Acad. Sci*. **859**, 75–84.

Hofer D, Shin DW, Drenckhahn D (2000) Identification of cytoskeletal markers for the different microvilli and cell types of the rat vomeronasal sensory epithelium. *J. Neurocytol*. **29**, 147–156.

Iseki S (1991) Postnatal development of the brush cells in the common bile duct of the rat. *Cell Tissue Res*. **266**, 507–510.

Ishida H (1977) Fine structural study on the postnatal development of the rat tracheal mucosa with special reference to the brush cells. *Yokohama Med. Bull*. **28**, 123–147.

Jarvi OH, Keyrilainen O (1955) On the cellular structures of the epithelial invasions in the glandular stomach of mice caused by intramural application of 20-methylcholanthrene. *Acta Pathol. Microbiol. Scand. A* **111**, 72–73.

Jeffery PK, Reid L (1975) New observations of rat airway epithelium: a quantitative and electron microscopic study. *J. Anat*. **120**, 295–320.

Johnson FR, Young BA (1968) Undifferentiated cells in gastric mucosa. *J. Anat*. **102**, 541–551.

Kasper M, Hofer D, Woodcock-Mitchell J, et al. (1994) Colocalization of cytokeratin 18 and villin in type III alveolar cells (brush cells) of the rat lung. *Histochemistry* **101**, 57–62.

Kotrschal K (1991) Solitary chemosensory cells-taste, common chemical sense or what? *Rev. Fish. Biol. Fish*. **1**, 3–22.

Kotrschal K (1996) Solitary chemosensory cells: why do primary aquatic vertebrates need another taste system? *Trends Ecol. Evol*. **11**, 110–113.

Kugler P, Hofer D, Mayer B, Drenckhahn D (1994) Nitric oxide synthase and NADP-linked glucose-6-phosphate dehydrogenase are co-localized in brush cells of rat stomach and pancreas. *J. Histochem. Cytochem*. **42**, 1317–1321.

Leeson TS (1961) The development of the trachea in the rabbit, with particular reference to its fine structure. *Anat. Anz*. **110**, 214–223.

Luciano L, Reale E, Ruska H (1968a) On a 'chemoreceptive' sensory cell in the tachea of the rat. *Z. Zellforsch. Mikrosk. Anat*. **85**, 350–375.

Luciano L, Reale E, Ruska H (1968b) On a glycogen containing brush cell in the rectum of the rat. *Z. Zellforsch. Mikrosk. Anat*. **191**, 153–158.

Luciano L, Reale E (1969) A new cell type ('brush cell') in the gall bladder epithelium of the mouse. *J. Submicrosc. Cytol*. **1**, 43–52.

Luciano L, Reale E, Ruska H (1969) Brush cells in the alveolar epithelium of the rat lung. *Z. Zellforsch. Mikrosk. Anat*. **95**, 198–201.

Luciano L, Reale E (1979) A new morphological aspect of the brush cells of the mouse gallbladder epithelium. *Cell Tissue Res*. **201**, 37–44.

Luciano L, Reale E, von Engelhardt W (1980) The fine structure of the stomach mucosa of the Llama. II. The fundic region of the hind stomach. *Cell Tissue Res*. **208**, 207–228.

Luciano L, Castellucci M, Reale E (1981) The brush cells of the common bile duct of the rat. This section, freeze-fracture and scanning electron microscopy. *Cell Tissue Res*. **218**, 403–420.

Luciano L, Reale E (1990) Brush cells of the mouse gallbladder. A correlative light- and electron-microscopical study. *Cell Tissue Res*. **262**, 339–349.

Luciano L, Reale E (1992) The 'limiting ridge' of the rat stomach. *Arch. Histol. Cytol*. **55**, 131–138.

Luciano L, Armbruckner L, Sewing KF, Reale E (1993) Isolated brush cells of the rat stomach retain their structural polarity. *Cell Tissue Res*. **271**, 47–57.

Luciano L, Reale E (1997) Presence of brush cells in the mouse gallbladder. *Microsc. Res. Techn*. **38**, 598–608.

Luciano L, Groos S, Reale E (2003) Brush cells of rodent gallbladder and stomach epithelia express neurofilaments. *J. Histochem. Cytochem*. **51**, 187–198.

Merigo F, Benati D, Tizzano M, Osculati F, Sbarbati A (2005) α-gustducin immunoreactivity in the airways. *Cell Tissue Res*. **319**, 211–219.

Meyrick B, Reid L (1968) The alveolar brush cell in rat lung – a third pneumonocyte. *J. Ultrastruct. Res*. **23**, 71–80.

Monteiro-Riviere NA, Popp JA (1984) Ultrastructural characterization of the nasal respiratory epithelium in the rat. *Am. J. Anat*. **169**, 31–43.

Nabeyama A, Leblond CP (1974) Caveolated cells' characterized by deep surface invaginations and abundant filaments in mouse gastro-intestinal epithelia. *Am. J. Anat*. **140**, 147–165.

Podkowa D, Goniakowska-Witalinska L (2002) Adaptations to the air breathing in the posterior intestine of the catfish (*Corydoras aeneus*: Callichthyidae). A histological and ultrastructural study. *Folia Biol. (Krakow)* **50**, 69–82.

Rhodin J, Dalhamn T (1956) Electron microscopy of the tracheal ciliated mucosa in rat. *Z. Zellforsch. Mikrosk. Anat*. **44**, 345–412.

Rhodin J (1959) Ultrastructure of the tracheal ciliated mucosa in rat and man. *Ann. Otol. Rhinol. Laryngol*. **68**, 964–974.

Rhodin JA (1966) The ciliated cell. Ultrastructure and function of the human tracheal mucosa. *Am. Rev. Respir Dis*. **93** (Suppl.), 1–15.

Riches DJ (1972) Ultrastructural observations on the common bile duct epithelium of the rat. *J. Anat*. **111**, 157–170.

Sato A, Miyoshi S (1996) Tuft cells in the main excretory duct epithelia of the three major rat salivary glands. *Eur. J. Morphol*. **34**, 225–228.

Sato A, Miyoshi S (1997) Fine structure of tuft cells of the main excretory duct epithelium in the rat submandibular gland. *Anat. Rec*. **248**, 325–231.

Sato A, Suganuma T, Ide S, Kawano J, Nagato T (2000) Tuft cells in the main excretory duct of the rat submandibular gland. *Eur. J. Morphol*. **38**, 227–231.

Sbarbati A, Crescimanno C, Benati D, Osculati F (1998) Solitary chemosensory cells in the developing chemoreceptorial

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epithelium of the vallate papilla. *J. Neurocytol*. **27**, 631–635.

Sbarbati A, Crescimanno C, Bernardi P, Osculati F (1999) alfa-gustducin-immunoreactive solitary chemosensory cells in the developing chemoreceptorial epithelium of the rat vallate papilla. *Chem. Senses* **24**, 469–472.

Sbarbati A, Merigo F, Benati D, et al. (2004) Identification and characterization of a specific sensory epithelium in the rat larynx. *J. Comp. Neurol*. **475**, 188–201.

Silva DG (1966) The fine structure of multivesicular cells with large microvilli in the epithelium of the mouse colon. *J. Ultrastruct. Res*. **16**, 693–705.

Sugimoto K, Ichikawa Y, Nakamura I (1985) Peroxidase activity in the epithelium of the digestive tract of the bullfrog *Rana catesbeiana*. *J. Exp. Zool*. **233**, 209–219.

Taira K, Shibasaki S (1978) A fine structure study of the nonciliated cells in the mouse tracheal epithelium with special reference to the relation of 'brush cells' and 'endocrine cells'. *Arch. Histol. Jpn* **41**, 351–366.

Thurlbeck WM (1990) A rose is a rose is a rose, but what is a brush cell? *Pediatr. Pulmonol*. **8**, 3.

Wada R, Watanabe T, Nakagawa K, et al. (2000) Structure of lamina propria lymphoid follicles and associated epithelium in the gastric mucosa during *Helicobacter pylori* infection in ulcer-bearing Mongolian gerbils. *J. Submicrosc. Cytol. Pathol*. **32**, 159–167.

Watson JH, Brinkman GL (1964) Electron microscopy of the epithelial cells of normal and bronchitic human bronchus. *Am. Rev. Respir. Dis*. **90**, 851–866.

Wattel W, Geuze JJ (1978) The cells of the rat gastric groove and cardia. An ultrastructural and carbohydrate histochemical study, with special reference to the fibrillovesicular cells. *Cell Tissue Res*. **186**, 375–391.

Wattel W, Geuze JJ, de Rooij DG (1977a) Ultrastructural and carbohydrate histochemical studies on the differentiation and renewal of mucous cells in the rat gastric fundus. *Cell Tissue Res*. **176**, 445–462.

Wattel W, Geuze JJ, de Rooij DG, Davids JA (1977b) Renewal of mouse gastric mucous cells following fast neutron irradiation. An ultrastructural and carbohydrate histochemical study. *Cell Tissue Res*. **183**, 303–318.

Whitear M (1992) Solitary chemoreceptor cells. In *Chemoreception in Fishes* (ed. Hara, TJ), pp. 103–125. New York: Chapman & Hall.

Wille KH (2001) The functional morphology of the large intestinal mucosa of the ox (*Bos primigenius* f. *taurus*), sheep (*Ovis ammon* f. *aries*) and goat (*Capra aegagrus* f. *hircus*). *Anat Histol. Embryol*. **30**, 65–76.

The executive summary of a workshop on 'Brush cells in the respiratory tract and other organs' can be found at http://www.nhlbi.nih.gov/meetings/workshops/brush-cell.htm

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