Anatomy and Cellular Constituents of the Human Olfactory Mucosa: A Review

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Abstract

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Studies using animal models have recently suggested that the olfactory mucosa may be a source of cells capable of stimulating and contributing to complex neurologic regeneration. Several groups have already transplanted cell derivatives from the olfactory mucosa into injury models, and the results so far have been promising. To fully appreciate the meaning of these experiments, a better understanding of the cellular biology and physiology of the olfactory system is necessary. It is therefore of utmost importance for us to first identify and understand its constituents.

Introduction

The olfactory mucosa is the part of the nasal mucosa that carries the specialized sensory organ for the modality of smell. Its structure, function, and physiology are unique yet poorly understood. Diseases associated with the olfactory system lead to an array of complex secondary presentations that are also not well understood including complaints of quality of life, mood changes, and depression.[1]

It is well known that the olfactory system is one of the two areas in the central nervous system (CNS)—the other being the hippocampus—where neuronal regeneration occurs. Yet in the clinical setting, the physician is still unable to exploit this exceptional characteristic for the patient's benefit. Furthermore, the lack of understanding of the olfactory mucosa renders the differential diagnosis for anosmia to range from entorhinal disease to complex CNS disorders such as Parkinson disease and Alzheimer disease. A better understanding of the olfactory mucosa is necessary for olfactory function to translate into accurate, useful clinical indicators for pathologic processes.

The olfactory mucosa has recently come under scrutiny as a potential source for cells that might be used for human tissue repair, and several groups have transplanted cell derivatives of the mucosa into injury models, often without really understanding the true constituents of the mucosa and transplant. Olfactory ensheathing cells in particular have attracted much attention, due to promising results in animal models of spinal cord repair[2,3] and the preliminary confirmation of safety in patients.[4] However, it is important to better understand the cellular components of cell cultures derived from the mucosa, and this in turn requires a better understanding of the olfactory mucosa itself.

Clinical and Developmental Anatomy

Gross and Clinical Anatomy

Upon gross examination, the human olfactory mucosa appears slightly yellow and without a distinctive hue as seen in rodents. It is generally agreed that it is located high in the nasal cavity, and specifically, it has been suggested to be concentrated in the posterosuperior aspect of the nasal cavity, in the two clefts formed superiorly by the cribriform plate, medially by the nasal septum, and laterally by the superior meatus.[5] Laterally, it can also be found in the posterior aspect of the superior turbinate and as far anteriorly as above and below the middle turbinate.[6,7]

In the clinical setting, gross examination of the olfactory mucosa may lack diagnostic value. First, the distribution of the olfactory mucosa is heterogeneous, thus making it difficult to distinguish from respiratory mucosa.[8] Second,

throughout life, respiratory epithelium gradually expands and replaces olfactory epithelium (OE).[8] And third, the olfactory tissue possesses multiple pathways for defense and regeneration[9] that makes morphological evaluation at any one point in time uninformative of overall mucosal health. The retrospective study conducted by Holbrook et al strongly implicated that rather than evaluating the morphological status of the OE, it is much more useful to examine the health of olfactory axons to determine the overall olfactory function.[10]

Developmental Anatomy

The OE is formed by two olfactory placodes that are ectodermal thickenings in the rostrolateral region of the embryonic head.[11–16] In humans, the OE is well delineated by day 37 postconception (pc).[17] The olfactory receptor neurons (ORNs) undergo ciliogenesis during 9 weeks pc, and by 11 weeks pc, there is complete morphological differentiation of ORNs and development of Bowman glands.[18] Olfactory marker protein (OMP) can first be detected in ORNs at 24 weeks pc[19] and in the olfactory bulbs (OBs) at 32 weeks pc,[20] but it has been reported that preterm infants born at 29 weeks pc exhibit clear behavioral responses to strong odorants.[21,22] In rodents, OMP is generally accepted as a marker of maturity due to its presence in differentiated ORNs,[23,24] but this criterion is obviously unsuitable for humans. The OE continues to develop after organogenesis and becomes morphologically identical to adult epithelium at late gestation.[25]

The physiology of fetal OE is worth mentioning here. Gu et al[26] found high levels of cytochrome P450 enzymes in the olfactory mucosa of developing human fetal tissue. High expression of these enzymes suggests that the olfactory mucosa may be deeply and intricately involved in the metabolism of maternally derived compounds; specifically, the olfactory mucosa may be a preferred target of certain toxic compounds. The recent quantitative analysis of the spatial distribution of UDP-glucuronosyltransferases, enzymes involved in detoxification, in mouse neuro-olfactory tissue further implies the role of OE in detoxification.[27] In addition to playing important metabolic roles, fetal olfactory tissue has been implicated to have immunologic functions by the discovery of Iba1- and annexin A3-immunopositive cells in the peripheral olfactory nerves of adult rats and adult cats.[28] Iba1- and annexin A3-immunopositivity suggests the presence of microglia/macrophages. These cells may be important in immunologic protection of the brain from infectious and toxic agents.[28]

Histology

The tissue lining the nasal cavity is composed of four types of epithelium. From outermost to innermost, they are (1) stratified squamous epithelium with numerous hair follicles, (2) transitional, cuboid, or columnar epithelium with no hair follicles, (3) ciliated pseudostratified columnar epithelium, and finally, (4) respiratory epithelium that consists of ciliated columnar cells, mucus-secreting goblet cells, and small basophilic cells believed to be stem cells.[29–31] Upon microscopic

examination, one finds that healthy olfactory mucosa is generally thicker and more cellular than respiratory mucosa.[32]

The olfactory mucosa is composed of three primary components: epithelium, basement membrane, and lamina propria. Adult OE can be identified using antibodies against trace amine-associated receptors and OMP; developing epithelial markers include epidermal growth factor receptors, transforming growth factor α, and nerve growth factor β.[33,34] The basal lamina, or basement membrane, lies beneath the epithelium and is usually a well-defined homogeneous structure.[35] On the other side of the basement membrane lies a thick lamina propria that contains mucous and serous cells, nerve fascicles, pigment cells, lymphoid cells, and blood capillaries (►Fig. 1).[36–38]

Before moving on to the discussion of different cell types, one other tissue structure worth specific mention is the olfactory pit, formed from invagination of OE into the underlying connective tissue. These structures vary from 150 to 200 μm in depth and 50 to 100 μm in diameter.[39] They are hypothesized to prolong odorant association with receptors by creating a pouched environment or to provide specific niches for specialized neurons that have yet to be discovered.[39] Like Bowman glands, olfactory pits are confined to the OE and are thus useful markers for distinction from respiratory epithelium.[40]
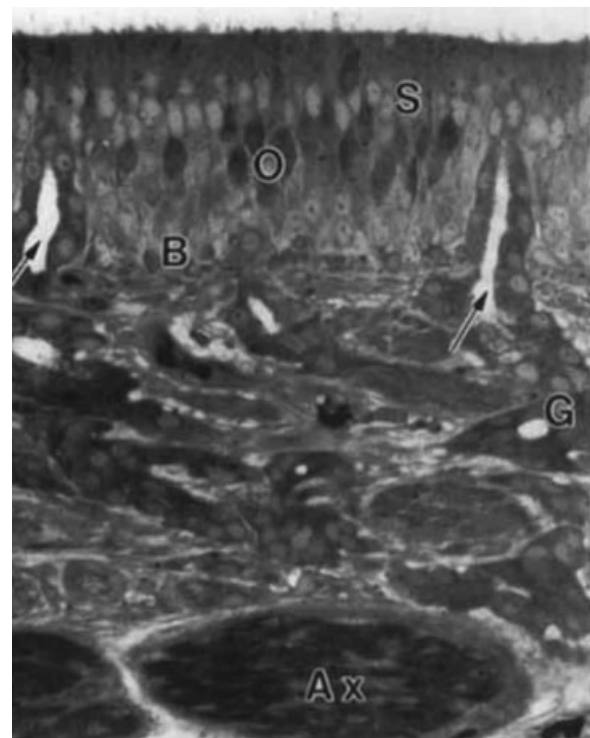
Fig. 1 Light micrograph thick section (1 μm) of human olfactory mucosa. The olfactory epithelium contains supporting (S), receptors (O), and basal (B) cells. The lamina propria contains olfactory axon fascicles (Ax) and Bowman glands (G), whose secretory ducts open to the mucosal surface (arrows). ×450. (Reprinted with permission from Morrison EE, Costanzo RM. Morphology of olfactory epithelium in humans and other vertebrates. Microsc Res Tech 1992;23(1):49–61.)

Cell Types in the Olfactory Mucosa

Epithelium

The OE is composed of five principal cell types: olfactory receptor neurons, sustentacular cells, basal cells, microvillar cells, and fingerlike microvilli cells.[30,41–44]

Olfactory Receptor Neurons

It is well known that ORNs are sensory cells specialized for detecting odorants. In humans, ORNs can be found at various stages of maturity and are interspersed with sustentacular cells.[45] The nuclei of ORNs are elliptical and usually darkly stained, the cell bodies are round or oval, ~ 4 to 6 µm in diameter, and the dendrites ascend in between sustentacular cells to terminate in a knob-bearing olfactory cilia, with each receptor cell having 10 to 20 cilia.[46–48] Gap junctions are present between ORNs, and they are believed to play a role in facilitating the development and turnover of tissue.[49] The morphology of the knob is flat or dome-shaped, not bulb-like. And cilia lie in the long axis of the olfactory cell, perpendicular to the epithelial surface rather than parallel as described in some nonhuman species.[41] Olfactory knobs and cilia in a nonparallel orientation under electron microscopy can be used to determine the presence of ORNs within the OE.[6]

Olfactory sensory cells can be marked by growth-associated protein-43),[50] β-tubulin 3,[6,51,52] MAP-1B,[53] neuron-specific enolase,[54] neurofilament protein,[54] neural cell adhesive molecule (NCAM),[55] neuron-specific tubulin,[56] PGP 9.5,[57,58] and OMP.[59–61] MAP-1B and NCAM identify dendrites and axons of ORNs and label nerve bundles intensely.[53] Carnosine-like immunoreactivity has also been demonstrated in ORNs.[62]

Sustentacular Cells

Sustentacular cells are irregular columnar cells with large vertically elongated euchromatic nuclei and multiple long microvilli.[41,63] Most sustentacular cells lie superficial to the soma of ORNs, and their cytoplasm contains many mitochondria, granular and agranular endoplasmic reticulum.[63] Many findings suggest that sustentacular cells play an important role in the regulation of ORN homeostasis and proliferation including the discovery of tight junctions between sustentacular cells and ORNs and complex calcium signaling in mouse sustentacular cells.[31,46,64]

SUS-1 and SUS-4 have been described as useful antibodies for labeling sustentacular cells in the rat.[65,66] Using electron microscopy, Pixley et al[67] found that 1F4, an immunoglobulin (Ig)M kappa monoclonal antibody, selectively labeled the microvilli of sustentacular cells and ductal cells of Bowman glands in the rat. They also bind to the microvilli and cilia of ciliated but not secretory cells in the respiratory epithelium.[67] More recently, Minovi et al[68] found that nestin expression is constantly detectable in the apical protuberances of sustentacular cells in healthy adults. But in the decline of olfactory function, nestin expression can be decreased.[68] These results suggest the possibility of nestin and 1F4 as markers for sustentacular cells and indicator of OE.

Bowman glands

It is known that Bowman glands are branched tubuloalveolar structures that lie beneath the OE and secrete onto the epithelial surface through narrow ducts.[31] In addition to bathing the dendritic endings and cilia of ORNs, thus allowing odorant diffusion to sensory receptors, the secretion is suggested to play important immunologic functions. Constituents of the secretory immune system including IgA, IgM and J chain, have been localized in the acinar and duct cells of Bowman glands and in the mucociliary complex.[69] Lactoferrin and lysozyme, two antimicrobial proteins, have also been found.[69]

Olfactory ensheathing cells

Olfactory ensheathing cells were first described by Golgi and by Blanes when they observed the glial populations in the olfactory bulbs of mammals.[70,71] In addition to residing in the first two layers of the OB, these cells are found in the OE, where the mesaxon of each ensheathing cell encloses densely packed bundles of unmyelinated axons (fila olfactoria) projecting from ORNs to the OBs.[12,14,72–76] Although OECs are found in the interstices between glomeruli in the OB, their processes never extend into the glomeruli.[72,77]

As mentioned previously, the OE develops from the olfactory placodes. The olfactory bulbs, in contrast, develop from the neural tube. Because of the dual developmental origin of the structures containing OECs, the origin of OECs is still debated. There is increasing evidence today that the OECs develop from the olfactory placodes. As they mature, OEC progenitor cells accompany ORN axons toward the OB by following a gradient created by soluble factors secreted by the target tissue. This idea is further supported by the nonimmunopositivity of OECs for A4 antibody.

Morphologically, olfactory ensheathing cell progenitors can be clearly distinguished by their dark round appearance, mode of association with axons, and ultrastructural characteristics.[14–16,73,78] Their lobulated nucleus contains patchy chromatin beneath the nuclear envelope and one or two nucleoli. As they mature, they acquire an elongated morphology with thin laminar processes that enfold small axonal bundles.[16] In the adult, OECs have a fusiform morphology with the perikarya aligned along olfactory fascicles.[73,78] Their nuclei are indented with uniformly distributed, yet slightly clumped chromatin below the nuclear membrane. In the cytoplasm, free ribosomes and large inclusion bodies are abundant. In comparison with astrocytes, OECs are electron denser and have intermediate filaments that are scattered rather than arranged in bundles.[74,79,80] The plasma membrane at the terminal ends of processes also lack the orthogonal arrays of intramembranous particles that are observed in those of astrocytes.[73,74,76,81,82]

To identify OECs, numerous markers have been suggested: platelet-derived growth factors,[83] neuropeptide Y,[84] S100,[85,86] glia-derived nexin (a neurite-promoting molecule),[87,88] L1 (cell adhesion molecule),[89] laminin,[90–92] polysialic acid-containing molecule,[93] NCAM,[89] and

p75NGFR. The expression of p75NGFR is stronger in neonatal OECs and almost undetectable, but not absent, in the adult.[86,93–95] Also, it has been found that NCAM and L1 are exclusively present in the part of glial membrane that associates with axons.[89] To distinguish OECs from Schwann cells, Boyd et al[96] reported that OECs exclusively express calponin. Tomé et al,[97] however, found calponin to be heterogeneously expressed by neonatal mucosal connective tissue but not neonatal OECs, embryonic OECs, and neonatal Schwann cells.

In recent years, olfactory ensheathing cells (OECs) have received much attention from the scientific community due to their application in regenerative medicine. It was suggested a few years ago that OECs from OE differ from those obtained from olfactory bulbs.[98] Notably, OECs from olfactory mucosa overexpress genes characteristic of wound healing and regulation of extracellular matrix, whereas OECs from olfactory bulbs express genes suggestive of nervous system development.[98] Within the population of OECs from olfactory bulbs, two subpopulations different in biophysical property and gap junction connectivity have also been found.[99]

In vivo, OECs form a matrix of cellular projections surrounding axons, unique among glia, and express high levels of connexin-43.[99] In the transitional zone between the peripheral nervous system and CNS, OECs have been found to interact freely with astrocytes and not to induce astrocytosis, which is a major difference between OECs and Schwann cells.[100,101] This property has been suggested to contribute to the ability of OECs to promote neural regeneration.[100] It has recently been found that OECs also secrete neurotrophic factors (e.g., NGF 74 and 75) that promote neurite growth.[102,103]

Basal Cells

It is believed that steady loss and replacement of ORNs and sustentacular cells throughout life is a normal process, and basal cells that lie above the basement membrane are the stem cells in the OE that divide to give rise to new neural and supporting cells.[104–107] In the rat it is well known that there are two basal cell types, (small) globose basal cells (GBCs) and horizontal basal cells (HBCs), and that mature and immature ORNs are organized in a highly laminar fashion with mature cells closer to the apical surface. In the human, however, there appears to exist only one basal cell type that morphologically resembles the GBCs in the rat.[53] These cells are usually 5 to 7 μm in diameter and have a rough cellular surface upon examination by electron microscopy.[46]

Intermediate filament proteins have been markers of interest in the past. It was previously assumed that nestin, one type of intermediate filament protein, was a specific marker for OE stem cells.[108,109] Using a bank of antibodies, Doyle et al[110] found that nestin is actually expressed in the axonal ends and inferior processes of OE sustentacular cells in the basal compartment of the epithelium. Hahn et al[53] further investigated the expression of cytokeratin-5, another class of intermediate filament proteins, in human OE, but disappointingly, the staining showed that cytokeratin is expressed not

only in the first layer of basal cells closest to the basal lamina but also in the cells above them.

It has been suggested that Ki-67, a cell cycle marker, be used as criterion for putative neural precursors in human OE.[53] The limitation of this marker is that 20% of the labeled cells reside in layers above the basal one, and not all cells positive for Ki-67 are positive for p75NGFR, a protein known to be expressed in basal cells as well as OECs.[53,111,112] Currently, there is still no known marker that exclusively labels human OE stem cells.

Whether HBCs or GBCs are the stem cells in the rat that give rise to the other or to the neural and nonneural cells in the OE has been a subject of debate.[104,113–118] The following section reviews the histology and proposed function of these two cell types.

Horizontal Basal Cells

Horizontal basal cells (HBCs) lie deepest in the OE and closest to the basement membrane, and they maintain a flat morphology.[119] They are relatively quiescent and are thought to divide only occasionally to give rise to GBCs, which are assumed to then give rise to ORNs and sustentacular cells.[114,120,121] In the event of severe damage to the OE, HBCs have been found to divide more frequently to give rise to multiple cell types.[114,120] This also potentially accelerates tissue repair. It has been suggested that HBCs can give rise to OECs.[113]

Globose Basal Cells

Globose basal cells (GBCs) lie above the HBCs and have a rounder morphology.[119] They can be labeled using cytokeratin, p75NGFR, and GBC-1, a monoclonal antibody that exclusively labels GBCs.[66,68,122] In animal models, GBCs are found to be necessary for regeneration of OE after lesion.[123] Their ability to give rise to either neurons, nonneurons, or both cell types in the OE proves that they are multipotent cells.[124]

Microvillar Cells

Microvillar cells are located near epithelial surface.[42] Although they are flask-shaped with a tuft of blunt microvilli that extends into the mucus layer of the epithelium, a thin axon-like cytoplasmic process extends from the basal pole of these cells and travels through the epithelium toward the lamina propria, rendering a bipolar morphology.[42] Microvillar cells are positive for spot-35 proteins, a type of neuron-specific protein.[125] Experiments tracing the flow of enzymes further show these cells to be connected to the olfactory bulbs.[126] Microvillar cells may very well represent a second morphologically distinct class of chemoreceptors in the olfactory mucosa.[42,125,126] But it has also been demonstrated that a loss of microvillar cells does not affect olfactory function.[127]

Fingerlike Microvilli Cells

One report from Ota[44] described a fifth type cell in the OE, and this cell type was found only after the disappearance of the olfactory cilial mat following resection of the olfactory bulbs. Transmission electron microscopy observation reveals that

the microvilli of these cells are characterized by a specific core structure consisting of microfilament bundles absent in the microvillar cells. Observing a disconnection between these cells and the postganglionic fibers of the trigeminal nerve and the olfactory bulbs, the author suggests that the fifth type cell could be a mechanoreceptor for a sensory system that is nonolfactory.

Lamina Propria

The lamina propria of the olfactory mucosa contains numerous cell types and structures including endothelial cells that make up the blood vessels, Schwann cells that myelinate processes of sensory neurons, glandular cells of Bowman glands, and stem cells, which have become of significant interest in recent years.

Lamina propria–derived stem cells (LPSCs) have been shown to grow in large numbers and to differentiate into neural and nonneural cell types both in vitro and in vivo.[128] This is a feature not observed in neurosphere-derived stem cells.[129] Immunomarkers and flow cytometry also suggest that these cells have little in common with neural stem cells and hematopoietic stem cells.[129]

Studies have shown that LPSCs may have vast replicative potential because they can generate dopaminergic cells after transplantation in a rat model of Parkinson disease and can also give rise to mesodermal cell types.[128,130] For this reason, these residents of lamina propria have been referred to as mesenchymal(-like) stem cells.

Olfactory Mucosa in Culture

In explant cultures of human OE, two cell types are found to have p75NGFR immunoreactivity.[53] The first type is found to have a round to polygonal morphology, and they are immunopositive for Ki-67 and negative for glial fibrillar acidic protein (GFAP). These cells are hypothesized to be equivalent to OE basal cells. The other type is spindle-shaped and immunopositive for GFAP. Interestingly, these cells are found around the edges of the epithelial sheets that grow out of the explants.[53]

Cells positive for OMP have round or oval cell bodies and possess a bipolar morphology, as do in vivo ORNs. It has been demonstrated that ORNs in dissociated cultures can respond to odorant stimulation by changes in intracellular calcium even if their cellular morphologies appear immature.[131]

In vitro, neonatal human OECs from OB have been found to express the glial markers S100, GFAP, p75NGFR, ErbB1–2–3 receptors, but not ErbB4, and neuregulin (NRG)-1.[132] Depending on the isoform, NRG-1 can be found either in the nucleus or cytoplasm.[133]

The ultrastructure of OECs remains the same in vivo and in vitro, but in animal models, the morphology of OECs can vary tremendously depending on the age of the tissue donor and the presence or absence of serum in the culture medium.[74,134,135] When cultured from mouse embryos and grown in medium with serum, they appear flat, bipolar, or tripolar. In chemically defined medium, cells change from flat to bipolar spindly, tripolar, or stellate.[135] When cultured from neonatal rodent epithelium in serum-containing medium, most of the cells are flat with extended cytoplasm; the rest are bipolar or tripolar with long and thin processes.[74,136,137] Moving these cells to a serum-free media results in an increase of cells with a bipolar or multiprocess appearance.[137,138] Experiments using culture of OECs from rat OB have also been done. Most of these cells (> 94%) are flattened and exhibit a fibroblast-like morphology when cultured in serum-containing medium.[93,94] When moved to a serum-free environment, a new population with a spindly morphology emerges.[93,94]

Coculturing with neurons also changes the morphology of OECs, but the change depends on axonal contact and seems to be independent of age of tissue donor and culture conditions. In the presence of axonal contact, OECs acquire a bipolar spindly appearance and are able to ensheathe individual axons.[139–142] Interestingly, when cocultured with myelinated dorsal root ganglion neurons, OECs form myelin sheaths around the axons of these cells.[140]

Conclusion

The olfactory mucosa is a highly organized tissue, unique and fit for its purpose. It is possible to isolate many different types of cells including stem cells and olfactory ensheathing cells, and culture them for possible applications in tissue repair. However, it is clear that there are major differences in the numbers and types of cells that can be obtained from cultures of human and rodent samples of mucosa, and we need to study these differences more closely in in vitro tissue culture and in in vivo models. We need to study how cell culture yield changes with patient age, smoking, pollution, and the presence of chronic inflammatory diseases of the nose, as well as to validate culture to good manufacturing practices standards. The positive experimental models of repair using cell therapies are outside the scope of this article, but many show promise, and it is necessary therefore to turn the spotlight on the nature of the cells we are using for cellular therapies derived from olfactory mucosa.

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