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Neural Crest and Olfactory System: New Prospective

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Abstract Sensory neurons in vertebrates are derived from two embryonic transient cell sources: neural crest (NC) and ectodermal placodes. The placodes are thickenings of ectodermal tissue that are responsible for the formation of cranial ganglia as well as complex sensory organs that include the lens, inner ear, and olfactory epithelium. The NC cells have been indicated to arise at the edges of the neural plate/dorsal neural tube, from both the neural plate and the epidermis in response to reciprocal interactions Moury and Jacobson (Dev Biol 141:243–253, 1990). NC cells migrate throughout the organism and give rise to a multitude of cell types that include melanocytes, cartilage and connective tissue of the head, components of the cranial nerves, the dorsal root ganglia, and Schwann cells. The embryonic definition of these two transient populations and their relative contribution to the formation of sensory organs has been investigated and debated for several decades (Basch and Bronner-Fraser, Adv Exp Med Biol 589:24–31, 2006; Basch et al., Nature 441:218–222, 2006) review (Baker and Bronner-Fraser, Dev Biol 232:1–61, 2001). Historically, all placodes have been described as exclusively derived from non-neural ectodermal progenitors. Recent genetic fate-mapping studies suggested a NC contribution to the olfactory placodes (OP) as well as the otic (auditory) placodes in rodents (Murdoch and Roskams, J Neurosci Off J Soc Neurosci 28:4271–4282, 2008; Murdoch et al., J Neurosci 30:9523–9532, 2010; Forni et al., J Neurosci Off J Soc Neurosci 31:6915–6927, 2011b; Freyer et al., Development 138:5403–5414, 2011; Katoh et al., Mol Brain 4:34, 2011). This review analyzes and discusses some recent developmental studies on the OP, placodal derivatives, and olfactory system.

Keywords Olfactory placode · Neural crest · Olfactory ensheathing cells · Migratory mass · GnRH-1 neurons

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From the Olfactory Placode to the Migratory Mass

Placodes are focal areas of specialized tissue that undergo morphological changes, such as thickening and invagination, in response to environmental and intrinsic stimuli (Fig. 1). It has been proposed that all placodes are derived from the induction of a contiguous pre-placodal field of cells located around the anterior neural plate [1–3]. With respect to the olfactory placodes (OP), graft experiments in chicken and quail have shown that the OP does arise from cells along the anterior neural folds ([4] reviewed in [5]). However, fate-mapping studies in zebrafish and chicken that focused on pre-placodal stages, suggested that both olfactory and otic placodes arise from the recruitment of a large field of intermingled heterogeneous cells, including neural crest (NC) cells, that converge to form placodes in response to inductive signals [6, 7]. Evidence in mouse also indicates that these two cranial placodes are composed of cells of heterogeneous genetic lineages [8, 9]. In the OP of rodents, cells sharing common genetic lineage with the NC have been shown to differentiate into subsets of sensory neurons, support cells, cell of the respiratory epithelium, peptidergic neurons, and olfactory ensheathing cells (OECs; specialized

glia of the olfactory system) [8–11]. However, graft based studies in chicken [12, 13] suggest that although NC cells are responsible for the formation of the OECs, sensory neurons and peptidergic neurons solely derive from non-neural ectodermal progenitors of the anterior neural folds [12, 13]. So, are we any closer to understanding the cell lineage of the progenitors of sensory placodes?

The OP in mouse is detectable around 9.5 days of development (E9.5) and neuronal differentiation in the OP starts between E10 and 10.5 [14]. Shortly after placodal invagination, numerous cells delaminate [15] and migrate out of the placode to the nasal mesenchyme as neuronal projections start to extend from the invaginating placode to the developing telencephalon [16–18] (Fig. 1). The existence of cells migrating out of the nasal placode has been described over a century ago [16]. Later analysis in multiple species showed that the olfactory pit is a source of heterogeneous migratory cells, including neurons, glia, and proliferative progenitor cells [17, 19–27]. The migratory cells, together with the nascent axons, form the migratory mass (MM) [28]. The identity and destiny of only a subset of the postmitotic neuronal precursors [22, 29, 30] in the MM is known (Fig. 1a–k). These include peptidergic neurons expressing either neuropeptide-Y [31] or gonadotropic releasing hormone-1 (GnRH-1) [17, 27], with the latter forming the GnRH-1 neuroendocrine system that is essential for reproduction (review, [32]) (Fig. 1j, k). Olfactory ensheathing cells are associated with the nascent olfactory fibers and neurons of the MM and migrate toward the part of the developing forebrain that will later form the olfactory bulb [19, 22] (Fig. 1d, e, and h). The OECs can be genetically traced [33, 34] and visualized using mouse models expressing Cre recombinase and or reporter genes under the control of promoters such as Sox10 or BLBP (Fig. 1l, m) [33, 35]. It has been suggested that cells of the MM play a role in perforation of the basal lamina of the OP [22], guiding neuronal projections from the placode to the forebrain, and influencing the induction and development of the olfactory bulb [19, 36–39]. However, the developmental role of the different cells of the MM and their final destination once they have completed development is still only partially understood.

Between E10.5 and E11.5 in mouse, when the placode progressively invaginates forming the olfactory pit and MM, the composition of the pre-olfactory epithelium (OE) and surrounding mesenchyme is dramatically different from what is observed in the developed olfactory mucosa. At early stages, there is no lamina propria and mucus-producing (Bowman's) glands [14], differentiated sustentacular/support cells, and mitotic basal progenitors are absent [40] (see Fig. 2e for a schematic representation). In the invaginating OP, as described in other placodes, the mitotic progenitors are localized at the apical portion of the pit facing the lumen of the developing olfactory cavity [14, 15, 40–42]. Progenitor cells in the invaginating OP respond to environmental cues and are distinguishable based on their expression of molecular markers and proliferative ability [43]. BrdU birth date tracing experiments in mouse, as well as neurogenic marker tracing in chicken, have shown that a large portion of the postmitotic neuronal cells born in the first neurogenic divisions (E9.5 and E10.5 in mouse) are destined to become part of the MM rather than the OE [42, 44]. This suggests that the molecular cues at early phases of placodal development are distinct from those responsible for the formation of the olfactory sensory epithelium at later embryonic and postnatal stages.

Kallmann Syndrome, GnRH-1 Neurons, and GnRH-1 Cell Lineage

GnRH-1 neurons, a component of the MM, originate in the nasal area and migrate along axonal projections to the forebrain (Fig. 1j–k). These cells play a central role in controlling sexual development and reproduction via the hypothalamic-pituitary-gonadal axis [17, 27, 45]. The embryonic origin of GnRH-1 neurons has been a matter of debate for several decades. Studies on different animal models have provided compelling and contrasting evidence of placodal, nonplacodal ectodermal, and NC origins for these cells [8, 13, 27, 46–50] and thus are an important component in the debate on the origin and composition of the OP itself.

Morphological studies in mouse indicate GnRH-1 neurons originate from slow dividing progenitor cells in the Ap2a and Meis 1 expressing area at the border between the putative respiratory epithelium and developing vomeronasal organ [27, 42, 43, 51]. Postmitotic GnRH-1 precursors, in mouse, start to express the GnRH-1 peptide around E11.5 as they delaminate and migrate, along axonal projections, to the forebrain [27, 32, 42, 46, 52, 53]. Though morphological evidence points to the existence of GnRH-1 progenitors in the olfactory pit, it does not clearly define the embryonic lineage for these cells [7, 42, 50, 54]. Using genetic lineage tracing in mouse, it was confirmed that the majority, but not all, of GnRH-1 neurons originate from placodal ectodermal progenitors [8]. By contrast, recent cell fate tracing in chicken, based on surgical grafts of putative neural fold ectoderm versus NC, suggested exclusive placodal ectodermal origin for GnRH-1 neurons and no NC contribution to the OP in chicken [13]. However, in a variety of species (including chick), progenitor cells of putative NC origin have been identified within the OP [8–10, 50] and together with genetic lineage tracing in mice, indicate an additional NC contribution to the GnRH-1 population can occur [8].

Kallmann syndrome (KS) is a pathology associated with defects in development of the olfactory system and impaired migration of GnRH-1 neurons [55]. The clinical features of KS include total or partial anosmia (lack of smell) and lack

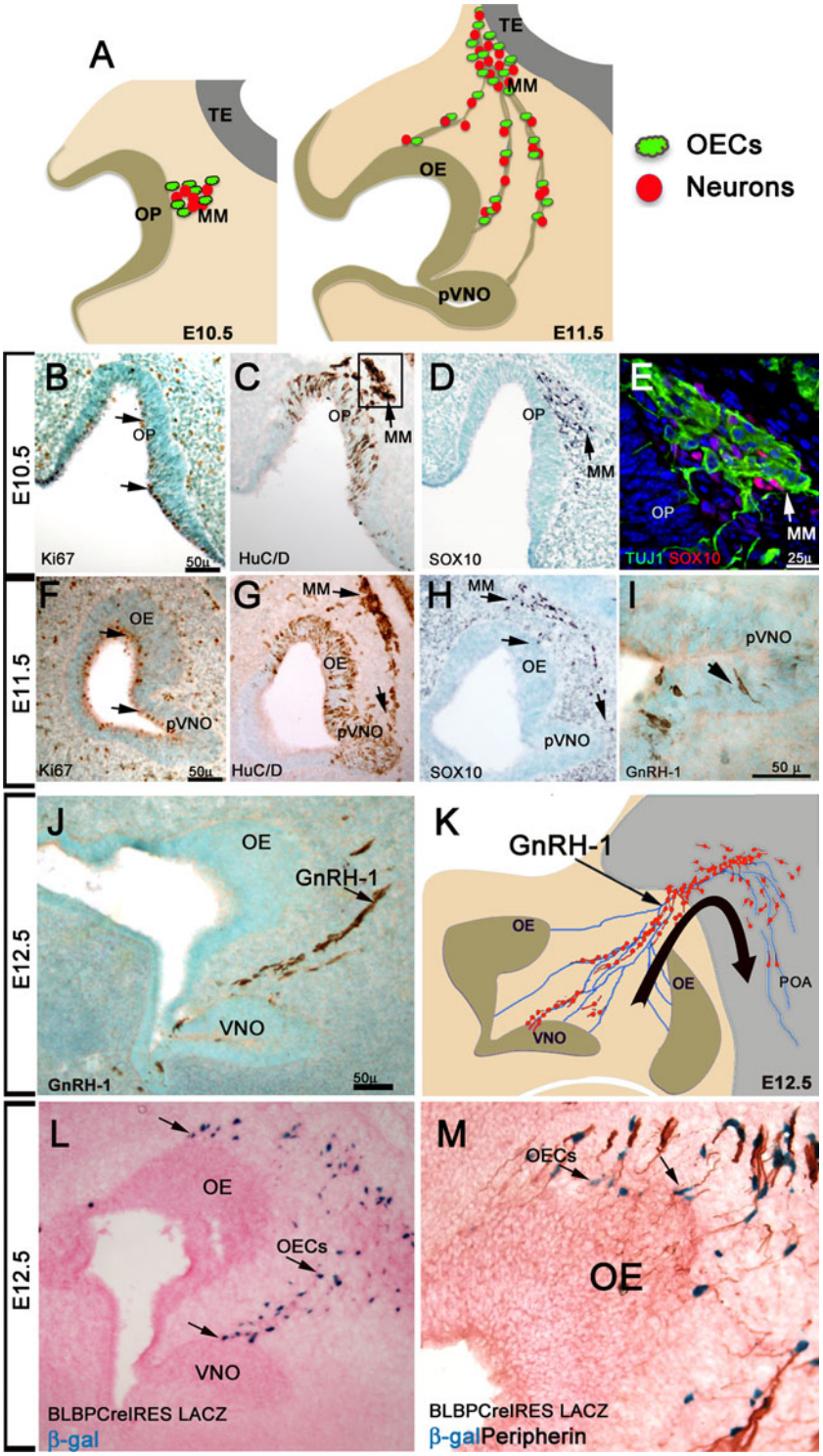

Fig. 1 Olfactory placode, olfactory pit and migratory mass. **a** Schematic showing olfactory placode (*OP*) and migratory mass (*MM*) at E10.5 and olfactory epithelium (*OE*), putative vomeronasal organ (*pVNO*), and MM and telencephalon at E11.5. **b**–**e** E10.5. **f**–**i** E11.5. **b**, **f** Ki67 immunostaining highlights proliferative progenitors in the OP and olfactory pit (*brown*, *arrows*). **c**, **g** HuC/D immunostaining labels neuronal precursors in the OP and MM at E10.5 (**c** *arrow*; *boxed*) and neurons in the OE, pVNO and along the MM at E11.5 (**g**, *arrows*). **d**, **h** Sox10 labels the glial component of the MM at E10.5, and glia distributed along the migratory path at E11.5, compare (**g**)–(**h**). **e** Tuj1/Sox10 double immunostaining for neurons (*green*) and glia (*red*) in the MM (area corresponds to box in (**c**) shows no co-expression. **i** Early GnRH-1 expressing neurons proximal to the putative VNO. **j** Immunolabeled GnRH-1 neurons migrating, as part of the MM, from the developing VNO to the forebrain. **k** Schematic illustrating the migration of GnRH neurons from the olfactory area to the preoptic area (*POA*) of the brain. **l** X-gal reaction on E12.5 section of BLBP CreIRES LacZ. OECs cells (*blue*) positive for BLBP expression distributed along the migratory path starting from the developing OE and VNO (*arrows*). **m** Peripherin staining and X-gal reaction on section of BLBP Cre IRES LACZ reveals OECs (*arrows*) associated with olfactory fibers starting from the OE

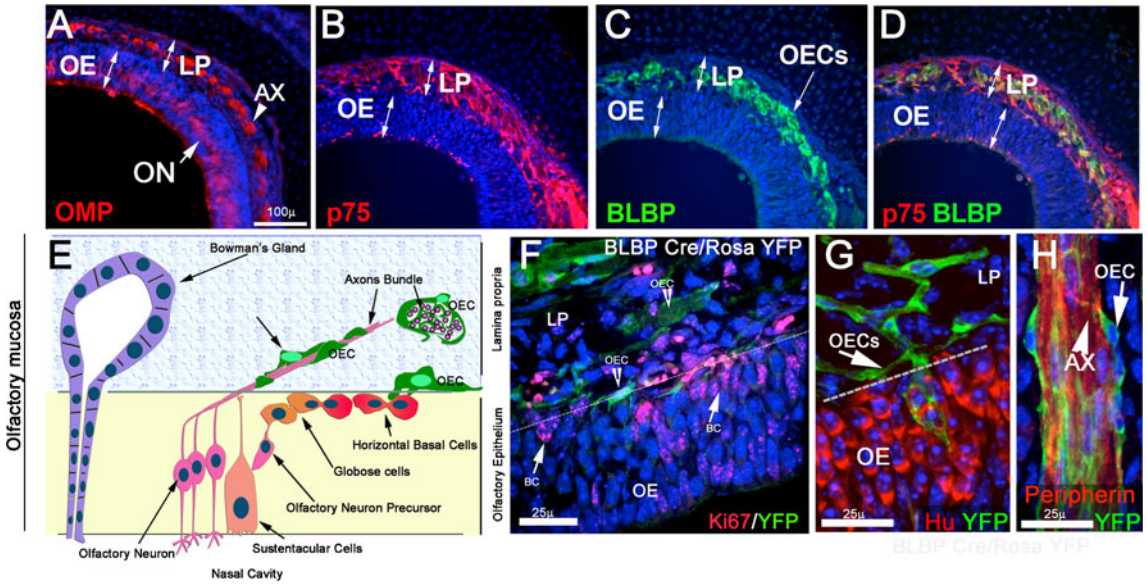

Fig. 2 Structure and cell composition of olfactory mucosa. E19.5 (**a**–**d**) OMP immunostaining (**a**) labels soma of olfactory sensory neurons in olfactory epithelium (*OE*) and olfactory axons in lamina propria (*LP*). **b** p75 immunostaining labels cells and connective tissue of the LP. **c** BLBP immunostaining labels olfactory ensheathing cells (*OECs*) in LP and at the basal portion of the OE. **d** Merged image of (**b**) and (**c**) showing OECs in LP. **e** Schematic of the cell composition of the olfactory mucosa. **f** Ki67/YFP double immunostaining on sections from BLBPCre/RosaYFP mouse highlights OECs in LP and proliferative OECs proximal to the proliferative basal progenitor cells of the OE. **g** Hu/YFP double immunostaining on BLBPCre/RosaYFP section shows OECs at the basal portion of the OE extend their cytoplasm around Hu+ olfactory neurons. **h** Olfactory ensheathing cells surrounding peripherin-positive olfactory axons bundles

of pubertal onset [32, 56, 57]. Though anosmia, which is usually associated with aplasia or hypoplasia of olfactory bulbs and tracts, and hypogonadism are the classic clinical features of KS, additional NC-associated defects have been reported, including craniofacial defects, cleft palate and lip, sensorineural defects, deafness, cerebellar ataxia, dental agenesys, abnormal kidney morphogenesis, heart defects, and coloboma [58–65]. The NC-associated defects in this syndrome are consistent with NC-derived cells contributing to both the GnRH-1 neuronal system as well as the developing olfactory system.

Thus far, only a few genes have been identified that play a role in controlling olfactory/GnRH-1 development and correlate with the etiology of KS in humans [47]. These include mutations affecting *Kal1* (Anosmin), fibroblast growth factor receptor 1 (*FGFR1*), fibroblast growth factor 8 (*FGF8*), prokineticin 2 and its receptor (*PROK2/PROKR2*), chromodomain-helicase-DNA-binding 7 (*CHD7*) [66, 67], and nasal embryonic LHRH factor [68] genes, which have been associated with the etiology of ~35% Kallmann cases [69].

Animal models indicate that genes involved in FGF8 signaling, such as *Kal1*, *FGFR1*, *FGF8*, and *CHD7*, are crucial for both placodal development and cell specification, as well as for NC formation, migration, and survival [70–75]. Due to the broad effects of the mutations identified thus far and the intimate relation between NC-derived nasal mesenchyme and olfactory placode development [76, 77], no clear picture emerges about cell autonomous effects and the hierarchy of molecular and cellular events underlying normal olfactory/GnRH-1 development.

The Olfactory Mucosa and the Stem Cell Puzzle

The mature olfactory system has a peripheral component, the olfactory mucosa, and a central component, the olfactory bulbs, which process and redirect peripheral inputs to the brain cortex (Fig. 2). The olfactory mucosa (Fig. 2) comprised the OE, which is the superficial layer, and the lamina propria, a layer of vascularized, NC-derived ectomesenchymal tissue juxtaposed to the OE. The OE is a pseudo-stratified epithelium composed of olfactory sensory neurons, sustentacular cells, olfactory progenitor cells, and Bowman's gland ducts. The acinus of the mucus-producing Bowman's glands and the OECs are located within the lamina propria (Fig. 2). Olfactory neurons project their axons from the OE to the brain, going across the lamina propria, connective tissue, and bones. Olfactory ensheathing cells wrap olfactory axons in bundles from the basal lamina of the OE to the olfactory bulb. The OE is in direct contact with the external environment and therefore exposed to a multitude of chemical and biological insults. Luckily, the OE retains a unique regenerative ability throughout life. As such, it is able to regenerate aged or damaged neurons as well as the full repertoire of non-neuronal cells, ensuring functional recovery and chemo-detection [78, 79]. Numerous lines of evidence support the existence of distinct

kinds of olfactory progenitors and stem cells in both the OE and lamina propria, which differ in molecular expression profiles, hierarchical lineage relationship, and the ability to differentiate into different cell types (potency). Do these studies help define the make-up of the OP?

Within the OE, two olfactory basal progenitor/stem cell types have been described: globose basal cells (GBCs) and horizontal basal cells (HBCs) [80–82]. Intrinsic cell features, such as receptors, transcriptional factors, and epigenetic factors are determinants in defining the potency of progenitor cells [43, 83]. GBCs and HBCs differ in terms of molecular expression. GBCs express Sox2, Pax6, and GB2 (globose cell marker) and, depending on their differentiation state, can also express ASCL-1/MASH1 (as transit amplifier progenitors) and tubulinIII [84]. HBCs also express Pax6 and Sox2 [85] and express TrKa, p75, NT4, cyto-keratin, EGFR, ICAM-1 (CD54), β1-integrin, and β4-integrin. In addition, α1- (CD49a), α3- (CD49c), and α6-integrins (CD49f) may be differentially expressed among HBCs [84, 86, 87]. Sustentacular cells, also express Pax6 and Sox2 [85] and are able to self-replicate, a potential stem role for this cells is still an open question [34].

HBCs have been described as having a high level of potency (multipotency). In fact, in vivo experiments based on genetic lineage tracing identified populations of HBCs able to regenerate the full repertoire of OE cells after lesion [88] and in vitro observations on clonally selected populations of HBCs proved that these cells can produce OECs in addition to epithelial cells [84]. While studies have identified both the cell types in, and the regenerative ability of olfactory progenitors in postnatal OE, there still is little known about the relationship between early embryonic olfactory progenitors, gene expression, and lineage of the olfactory progenitor/stem cells in postnatal/adult stages [34, 40, 43, 89–91]. Whether HBCs are a homogenous population or composed of multiple cell types is not fully understood, as there is yet no identified unique molecular expression pattern. The embryonic lineage of these cells is also still a matter of investigation [34, 84].

Studies seeking to isolate stem cells from olfactory mucosa for broad regenerative purposes have identified additional multipotent "stem" cells in the lamina propria of mouse, rat and human. These cells are able to generate a wide range of cell types such as bone, adipose, smooth muscle cells, glia and neurons [92–96]. The phenotypic potency of these cells to produce both neuronal and mesodermal cells is similar to that described for skin-derived precursors, which are believed to be of NC origin [97]. It has to be noted that ecto-mesenchymal derivatives of the head (cartilage, bone, and connective tissue), including those of the lamina propria, are in large part derived from the NC and NC subfields [76, 98–100]. Therefore, it is likely that multipotent NC- derived progenitors within the lamina propria of mammals retain the ability to acquire specific phenotypes, including olfactory cell lineages, in response to specific developmental or regenerative cues, and might function as additional stem cell reserves for the olfactory mucosa [10, 97, 101, 102]. This interpretation is consistent with the fact that several genetic lineage tracing studies using Cre-drivers able to recombine in NC (P0, Wnt1, and PAx7), have identified putative NC cells in the embryonic and adult OE and lamina propria [8–11].

NC Origin of OECs and Contribution to the OP; Convergence and Contradictions

The OECs are a specialized glial population of the postnatal olfactory system (Fig. 3). Mature OECs localize together with committed progenitors within the lamina propria and along the olfactory fibers projecting to the bulbs. When transplanted into lesioned spinal cord, OECs have the ability to myelinate axons like Schwann cells of the peripheral nervous system [37, 103, 104]. Like Schwann cells [105], OEC precursors express Sox10 and BLBP (brain lipid binding protein or fatty acid-binding protein 7) [22, 34, 42] and OECs can express the astrocyte "specific" marker (GFAP), the low-affinity p75 nerve growth factor receptor (p75 NGFr), S100, Erbb2, as well as adhesion molecules such as laminin and N-CAM. In rodents, the expression of the OEC markers GFAP, p75, and S100 has been reported in putative OEC precursors within the OE and along the axonal projection at later developmental stages [87, 106]. Based on heterogeneity in molecular expression and regenerative ability when transplanted in lesioned spinal cord, the existence of different OEC subpopulations have been hypothesized [107–111].

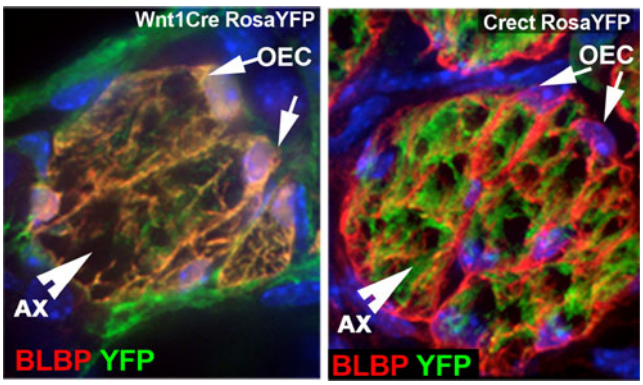

Fig. 3 Neural crest origin of the OECs, genetic lineage tracing. BLBP immunostaining (*red*) of OECs on sections from a Wnt1Cre/RosaYFP mouse (*left*) and Crect/RosaYFP mouse (*right*) highlights that OECs are positive for Wnt1Cre tracing and negative for Crect tracing (ectodermal) while most axons in the bundle are negative for Wnt1Cre tracing but positive for the ectodermal tracing

The similarity between OECs and Schwann cells (reviewed by Barnett [112]), in molecular marker expression, morphology, and cell behavior has puzzled the scientific community for decades [113–115] and suggested a potential common origin for these cell types [112, 116–120]. Traditional morphological studies, together with studies based on temporal gene/protein marker expression, had been unable to unravel the lineage and ontogeny of OECs. The OECs were initially believed to be peripheral Schwann cells [121] originating from NC precursors that migrated from the developing dorsal neural tube to the lamina propria, but a potential local origin from the placode was not excluded [122]. For the next two and half decades, evidence for a placodal origin of OECs increased, though NC was never totally excluded. These studies included:

1. Observations at early developmental stages of placodal invagination, when the lamina propria was not yet formed, [123],
2. Electron microscopy indicating OECs originated from olfactory basal cell progenitors [124],
3. Quail/chicken grafts of the pre-placodal anterior neural folds [4] and,
4. Isolation of multipotent stem cells from the OE of mouse with the ability to generate, OMP-, G_{olf}-, adenylate cyclase III-positive olfactory neurons, and cells positive for OEC markers GFAP, S100, and p75 [84, 125, 126].

However, recent reports have provided compelling evidence of a NC origin for these cells [8, 10–12, 127]. Lineage tracing in mouse, based on Pax7 expression [11], an early NC marker that is also expressed by a subset of cells in the anterior neural folds [128], first indicated distinct progenitor cells in the OP. Cells positive for Pax7 lineage were shown to give rise to a discrete population of neuronal and non-neuronal cells within the OE, subsets of neuronal cells of the MM and to the entire population of OECs [11]. These data suggested a potential NC contribution to the OP and NC origin for the OECs. A NC origin for OECs was demonstrated by anterior neural fold ectoderm grafts and by NC grafts in avian animal models [12]. In addition, two other studies in mouse, one using Wnt1Cre genetic lineage [12] and the other tracing placodal ectoderm and NC derivatives using a Cre ectodermal (Crect) mouse line as a superficial ectodermal tracer and Wnt1Cre mouse line as NC tracer [8], converged in defining the origin of OECs from NC- and not from ectodermal- progenitors.

Though these studies agreed about the NC origin of the OECs, some controversies arose. Barraud and coworkers showed, in avian models, that the OECs did not originate from grafted anterior neural fold ectoderm, which gives rise to the OP, but rather by grafted NC that also contributed to the formation of the nasal mesenchyme. Cells originating from the grafted NC were not detected in the placode. This suggested that OECs originate exclusively from NC progenitors that do not belong to the OE [12]. The graft experiments showed that the grafted NC were able to give rise to some of the p75-positive cells in the lamina propria and to P0-positive cells around the bulb. Notably, occasional OECs from anterior neural fold grafts (presumptive ectoderm) have also been described, but these cells are believed to derive from NC contamination of the grafted tissue [12]. These data suggested that the OECs originate from progenitor NC cells that (a) follow unknown routes to invade developing olfactory fibers as previously hypothesized [121] or (b) originate from differentiation of the nasal mesenchyme.

Lineage tracing in mouse [8] also demonstrated, that in mammals as in avian, the OECs were negative for ectodermal tracing (Crect) but positive for NC lineage (Wnt1Cre). In contrast to results described in chick, Forni et al. [8] suggested that multipotent cells of putative NC origin integrated in the OP, giving rise to OECs and contributing to the placodal-derived cell repertoire. This was based on the presence of progenitors positive for Wnt1 tracing in the OP, which was not observed by Barraud et al. [12], but confirmed by others [9, 10], and by complementary chimeric ectodermal (Crect) recombination in the OE and in placodal derivatives [8].

A potentially similar example of contrasting results in cell fate tracing obtained using similar paradigms (tissue grafts VS genetic tracing) has emerged in studies on the otic placode. Quail/chicken otic placode grafts indicated placodal origin for the inner ear though high levels of host/donor chimerism was observed in the inner ear after graft [129]. Yet, a recent genetic lineage tracing in mouse indicated that such chimerism might derive by the ability of NC cells to integrate in the developing otic placode and to contribute to the formation of the inner ear. These studies revealed a dual NC/ectodermal origin for this organ [9], as previously suggested for the neuromasts of the lateral line in fish [130]. So the question remains, for both the otic and olfactory placodes: are the interpretations biased by the technique?

Different Strategies and Different Outcomes

In graft experiments, portions of tissues of presumptive uniform cellular composition and cellular identity are topographically identified (relying on genetic expression maps at given developmental stages), mechanically excised and grafted into a host animal that has been deprived of the corresponding tissue. Though this technology has provided crucial information on topographic lineage of multiple tissues [4], it is not without problems. In fact, it does not allow one to establish if clonal cell heterogeneity existed prior to grafting the chosen tissue [7, 128]. In addition, the

substitution of the host tissue with the donor grafted tissue is often incomplete and consequently does not always permit a clear-cut discrimination between cell contamination from remaining host tissue and contributions of cells of other lineages [9, 12, 129]. Tissue graft tracing are intrinsically biased by a priori defining a temporal and topographic boarder and a clonal cell identity based on visual detectability of gene expression and previous fate maps.

Genetic lineage tracing relies on the identification of clonal contribution of progenitors with specific genetic features rather than on topographic localization, a key feature for tracing migratory/invasive cells such as the NC. Broadly described downsides of genetic lineage tracing are:

1. Potential ectopic [131] transgene expression,
2. Differences in the timing of transgene/endogenous gene expression, and
3. Partial penetrance and/or variability of recombination depending on the genetic mouse background [132].

So, one sees that both of these essential "lineage tracing" tools have caveats. However, are both interpretations of NC contributions to the OP possible?

Grafts and genetic lineage data may not necessary be mutually exclusive. The model of olfactory system development after grafts in avian [12], negates the existence of OEC progenitors within the OE [106] and implies that the previously identified olfactory multipotent progenitors isolated from OE [84, 124–126] might result from NC contamination. Support for differentiation of NC nasal mesenchyme to OEC cells, is the fact that mesenchymal cells are able to acquire a Schwann-like phenotype in response to unknown axonal inductive factors [133]. It is also possible that the p75- and P0-positive cells derived by NC grafts identified by Barraud and coworkers [12] could be derived from afferents of the peripheral nervous system. In fact, a contribution to the OEC population, could come from the ethmoid and nasopalatine branches of the trigeminal nerve, as trigeminal afferents to both OE, lamina propria and olfactory bulb have been described in multiple species [134–138].

On the other side, in support for the model proposed by Forni et al. [8] there is the following evidence. First, at E10.5 in mouse, OEC progenitors positive for Sox10 and Wnt1 Cre tracing appeared in a continuum, streaming out of the developing placode in association with the neurons of the MM [8]. Second, few olfactory axons, if any, are detectable at this early stage [22] making a connection between OE and the developing trigeminal nerve unlikely. Third, in vitro recombination experiments in mouse explants [77, 139] showed no mixing of frontonasal mesenchymal cells and presumptive placodal cells in the pit nor in the N-CAM expressing cells (neuronal precursors and OECs) of the migratory mass. Moreover, other independent NC genetic lineage tracing experiments in rodents based on the use of Wnt1cre line as well as other Cre animal models able to recombine in NC, such as Pax7Cre, P0Cre, and Pax3Cre, have confirmed recombination in the OECs as well as in progenitors within the OP and later on in the OE [9–11]. The convergence of these genetic tracing studies, together with the chimerism for ectodermal (Crect) tracing, make it unlikely that these observations are the result of recombination artifacts as suggested by others [13]. So how can one reconcile these different findings?

Possible interpretations that could reconcile the discrepancy between physical tissue grafts [12, 13] and NC genetic lineage experiments [8, 10, 11, 128] are:

1. At least part of the cells localized within the anterior neural folds, which are accepted as progenitors of the OP, share the same developmental potential of neural crest [140]; however, these cells do not usually undergo epithelial to mesenchymal transitions. The anterior neural folds, in which the expression of NC markers Snail1/2 is prevented by inhibition of Wnt/β-catenin signaling via Dkk1 [140], might be able to generate early subsets of progenitor cells that share common Pax7, Wnt1, P0, PAX3 genetic lineage and potency with the "classic" NC cells originating along the dorsal neural folds [141–144],
2. Stem cells able to give rise to OECs have been described and isolated from both OE and lamina propria [84, 125, 126, 145], if not simply resulting from mutual tissue contamination, would support the existence of NC progenitors in both regions, and
3. The results obtained by grafts in chicken and by genetic lineage tracing in mouse, if not species specific, represent two independent NC contributions to the olfactory system. Thus, it is possible that genetic tracing visualized an early developmental event in which a small NC population integrated into the developing pre-placodal tissues, contributing to the formation of placodal-derived neurons, and a second NC contribution, detected by graft experiments, gives rise to the nasal mesenchyme and glial cells in the olfactory system.

Conclusions

Sensory placodes and NC share a number of similarities: they both originate from specialized cells of ectodermal origin at the border of the neural plate. Both placodes and NC are able to generate cells with a plethora of different identities including sensory neurons and secretory cells. A potential common evolution for NC and placodes has been proposed [6, 146]. Based on evidence, proving the ability of the OP to give rise to glial cells and sensory neurons, the OP has for long time been believed to be the only placode able

to produce Schwann-like ensheathing glia [4, 124], a concept that found its constrain in the dogmatic vision of its exclusive ectodermal composition [7]. Based on this assumption, an overlapping developmental potential for placode and NC, to produce sensory neurons and glia, has been accepted until recent times.

The identification of a NC origin for the OECs indicates that all ensheathing glial cells of the peripheral system of the head, cranial ganglia, olfactory, and auditory system, are of NC origin. The established NC origin of the OECs and the identification of pluripotent NC-derived progenitors within the placodally derived structures [84] is an important point when considering the evolution of NC and placodal structures and more broadly the cellular and molecular readout of syndromic pathologies such as CHARGE and specific clinical cases of KS [71, 147].

The interaction between NC-derived cells and placodal-derived structures is intimate and crucial at multiple developmental levels. Neural crest-derived glial cells arising from the hindbrain are essential for the migration of epibranchial placode-derived neurons, and for the axonal targeting to the hindbrain [148]. The interaction between placodal and NC-derived components of the trigeminal ganglion is critical for both ganglion development and routing [149]. Various data indicate a key role for OECs in olfactory development, but thus far no specific animal models have been analyzed in this regard. OEC-specific ablation experiments in vivo and identification of genes responsible for OEC development are needed to unravel whether OEC development may underlie specific pathologies affecting olfactory and/or GnRH development.

The existence of progenitor cells in the OE with the ability to give rise to cells with different destinies, has been known for over a decade. Recent isolation and in vitro expansion of progenitors positive for NC tracing suggested these might be part of the previously identified multipotent progenitors [10, 84, 94, 150, 151]. Understanding the NC contribution to the olfactory system [7, 8, 50], is essential for re-interpretation of studies based on the use of olfactory-derived cells for regenerative purposes in the peripheral nervous system [111, 152–154]. Neural crest derivatives such as mesenchymal cells, Schwann cells, skin-derived NC progenitors and peripheral nervous system progenitors retain an extraordinary level of phenotypic plasticity in response of environmental cues [155–161]. The new findings suggesting that NC-derived cells can integrate in the developing sensory placodes and give rise to overlapping cell types as those of ectodermal origin [8–10] are exciting. This possibility provides a new scenario for understanding the biology and potency of NC cells and the role played by environmental milieus [155] in cell fate determination of cells deriving from progenitors of different embryonic origin.

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