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Restoring Olfaction: A View from the Olfactory Epithelium

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Damage to the olfactory periphery destroys the population of olfactory sensory neurons and, in the case of direct epithelial lesion, also eliminates other constituents of the epithelium. In marked contrast to other parts of the nervous system, there is substantial anatomical and functional recovery of the olfactory epithelium and its projection into the CNS even in the face of overwhelming injury. For example, in the case of epithelial lesions caused by a single exposure to methyl bromide (MeBr), the olfactory epithelium is restored to a status that is indistinguishable from unlesioned epithelium within 6–8 weeks after damage, despite the severity of the initial damage (>95% of the epithelium is destroyed). The first olfactory neurons reappear in large numbers on day 4 after MeBr, the first mature neurons emerge during week 2, and the accelerated production of neurons falls to normal around week 6 after lesion (Schwob *et al.*, 1995). The reconstitution of the epithelium is sufficiently robust and precise that the spatially restricted distribution of odorant receptor (OR) expression is also restored to normal (Iwema *et al.*, 2004). In this case, we assayed a set of eight different ORs, whose expression encompasses the whole of the tangential extent of the epithelium, by *in situ* hybridization (ISH). In rats in which the lesion caused by MeBr had been confined to one side of the epithelium by the temporary closure of a naris during the exposure to the gas, the boundaries of the expression territories of each of the eight were indistinguishable on the two sides. after recovery from the lesion (Iwema *et al.*, 2004). Remarkably, despite moderate damage to the GBC population, the spatial patterning of odorant receptor expression is restored, suggesting that extra-epithelial cues, perhaps emanating from deeper in the mucosa, might direct aspects of that patterning.

The projection of the epithelium onto the olfactory bulb is also restored quickly. By 2 weeks after lesion, axons fill the olfactory nerve layer and begin to re-enter the glomeruli. By 3 weeks, the glomeruli are densely innervated, and the projection becomes largely stabilized by 8 weeks (Schwob *et al.*, 1999). However, destruction of the neuronal population is not without consequence. Reinnervation of the olfactory bulb, although robust, does not fully restore the normally precise receptotopic organization of the projection. The newly generated axons target roughly the right part of the bulb, but often innervate multiple glomeruli in that locus as opposed to the usual one or two (C.L. Iwema, G. Ring and J.E. Schwob, unpublished data). Despite the degradation in the mapping function responsible for converting odorant stimuli into spatial patterns of neural activity, substantial function is restored after recovery from lesion (S.L. Youngentob, unpublished data).

In sum, the epithelium accomplishes a remarkable degree of functional as well as anatomical recovery after lesion.

How is that recovery accomplished? A series of investigations over the last few years has explored the capacity of the basal cell populations and the residual cells of Bowman's ducts to generate the various cell types that need reconstituting during the recovery process. Recall that there are at least two distinct populations of basal cells in the epithelium: horizontal basal cells (HBCs) and globose basal cells (GBCs) (Graziadei and Monti Graziadei, 1979; Holbrook *et al.*, 1995). The HBCs are highly differentiated cells that attach to the basal lamina by desmosomes, assemble cytokeratins 5 and 14 into intermediate filaments, enwrap bundles of olfactory axons as they exit the epithelium, and express a variety of other molecules in common with the basal cells of the respiratory epithelium. The GBCs are the small, round, morphologically non-descript cells that sit between the HBCs below and the immature OSNs above, that proliferate at a high rate in the normal OE, are limited to the OE, and are poorly characterized at the level of their molecular phenotype.

The studies have taken advantage of a series of cell type-specific antibodies, the use of replication-incompetent, retrovirally derived vectors (RRVV) for lineage tracing, and a colony forming unit assay which entails FACS isolation of potential progenitor populations followed by transplantation into a new host. The multiple, converging lines of evidence indicate that some among the residual GBCs function as broadly multipotent progenitors capable of giving rise to neurons and all of the cell types of the epithelium, and hence may be totipotent stem cells of the epithelium. First, marker studies using antibodies that are selective for GBCs in normal epithelium label cells that express both GBC and HBC markers, or both GBC and Sus cell markers, during the acute phase in the recovery after MeBr, suggesting that the GBCs are differentiating into these non-neuronal cell types (Goldstein and Schwob, 1996). Second, intranasal infusions of RRVV label GBC progenitors that give rise to neurons and multiple types of non-neuronal cells within the same clone, while other progenitors give rise to clones containing duct/gland cells and Sus cells (Huard *et al.*, 1998).

Third, and most directly, GBCs that are harvested from the normal epithelium, isolated by FACS using a GBC-selective antibody and free from HBC, duct/gland and Sus cell contaminants (to a level of <0.1% of total) engraft into the MeBr-lesioned epithelium after infusion into the nasal cavity and give rise to an impressive range of epithelial cell types (Goldstein *et al.*, 1998; Chen *et al.*, 2004). In aggregate, engrafted GBCs in the mouse can give rise to OSNs, Sus cells, BG/D cells, ciliated respiratory epithelial cells, and GBCs themselves (a form of self-renewal). Individual clones, derived from single engrafted GBCs, were composed of OSNs or Sus cells or OSNs, GBCs and Sus cells, or OSNs and BG/D cells or OSNs and respiratory epithelial cells (Chen *et al.*, 2004). In addition, clones that contain multiple cell types arise from GBCs that are infectable with a RRVV and hence mitotically active in the neurogenic epithelium. Moreover, the neurons that derive from the engrafted GBCs mature to the extent of making OMP, projecting axons to the olfactory bulb (specifically to the area of the bulb to which the surrounding host epithelium would project, and expressing an OR. Transplanted Sus cells give rise only to themselves. In contrast, HBCs either do not engraft i.e. in the mouse, or engraft but remain as HBCs, i.e. in rat.

In their totipotency and in their apparent self-renewal, the GBCs satisfy two of the several criteria that denote a tissue stem cell. Another indicant that at least some GBCs are bona fide tissue stem cells is that some among them are slowly cycling/quiescent and retain thymidine label for a prolonged period (X. Chen and J.E. Schwob, unpublished data). Finally, destruction of the GBCs eliminates the capacity of the epithelium to recover as olfactory after MeBr injury,

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forcing the epithelium to undergo respiratory metaplasia instead (Jang *et al.*, 2003).

The foregoing data indicate that GBCs are multipotent and probably stem cells, and that multipotent GBCs are making an active choice between making neurons and making non-neuronal cells in the normal, neurogenic epithelium. It appears that aspects of that choice are dictated by expression of members of the bHLH transcription factor family. Of the seven classes of bHLH markers, we have used ISH to study two of them: (i) transcriptional activators that drive neuronal differentiation, including *Mash1*, *Ngn1* and *NeuroD*, which are normally limited to GBCs; and (ii) repressors of neuronal differentiation that belong to the *Hes* group (Manglapus *et al.*, 2004). After MeBr lesion, the proneuronal factors reappear in a sequence that mimics their expression during the embryonic development of the olfactory epithelium (Cau *et al.*, 2002; Manglapus *et al.*, 2004): *Mash1* is eliminated from the epithelium the day after MeBr, but reappears at 2 days post-lesion in increased numbers. *Ngn1* and *NeuroD* also disappear and then re-emerge at 3 days post-lesion, in advance of the reappearance of large numbers of differentiating neurons 4 days after MeBr. In contrast, the neuronal repressor *Hes1* is expressed by Sus cells in the normal epithelium, but then materializes in GBCs 1 day after MeBr. Subsequently the *Hes1* (+) GBCs lose their association with the basal zone, shift apically at the surface of the thickening epithelium, and differentiate into Sus cells (Manglapus *et al.*, 2004). These data provide a molecular correlate for the differentiation of engrafted GBCs into Sus cells either alone or in combination with neurons (Goldstein *et al.*, 1998; Chen *et al.*, 2004). Given that Hes1 protein is known suppress transcription of *Mash1* (Davis and Turner, 2001), it appears that the residual GBCs are first driven to differentiate into Sus cells after lesion and only later do they begin to make neurons.

In conclusion, the remarkable capacity for anatomical and functional recovery by the olfactory periphery reflects the interplay between the GBC progenitors and the environment of the lesioned epithelium. Molecular signals control the choice made by the GBCs between making neurons vs. non-neuronal cells. In addition, cues from extra-epithelial tissue are likely responsible for directing which OR to express. The nature of the corresponding molecules and mechanisms remains a subject of ongoing investigation.

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References

Cau, E., Casarosa, S. and **Guillemot, F.** (2002) Mash1 *and* Ngn1 *control distinct steps of determination and differentiation in the olfactory sensory neuron lineage.* Development, 129, 1871–1880.

Chen, X., Fang, H. and **Schwob, J.E.** (2004) *Multipotency of purified, transplanted globose basal cells in olfactory epithelium.* J. Comp. Neurol., 469, 457–474.

Davis, R.L. and **Turner, D.L.** (2001) *Vertebrate hairy and Enhancer of split related proteins: transcriptional repressors regulating cellular differentiation amd embryonic patterning.* Oncogene, 20, 8342–8357.

Goldstein, B.J. and **Schwob, J.E.** (1996) *Analysis of the globose basal cell compartment in rat olfactory epithelium using GBC*-1, *a new monoclonal antibody against globose basal cells.* J. Neurosci., 16, 4005–4016.

Goldstein, B.J., Fang, H., Youngentob, S.L. and **Schwob, J.E.** (1998) Transplantation *of multipotent progenitors from the adult olfactory epithelium.* Neuroreport, 9, 1611–1617.

Graziadei, P.P.C. and **Monti Graziadei, G.A.** (1979) *Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons.* J. Neurocytol., 8, 1–18.

Holbrook, E.H., Szumowski, K.E.M. and **Schwob, J.E.** (1995) *An immunohistochemical, ultrastructural, and developmental characterization of the horizontal basal cells of rat olfactory epithelium.* J. Comp. Neurol., 363, 129–146.

Huard, J.M., Youngentob, S.L., Goldstein, B.J., Luskin, M.B. and **Schwob, J.E.** (1998) *Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells.* J. Comp. Neurol., 400, 469–486.

Iwema, C.L., Fang, H., Kurtz, D.B., Youngentob, S.L. and **Schwob, J.E.** (2004) *Odorant receptor expression patterns are restored in lesion-recovered rat olfactory epithelium*. J. Neurosci., 24, 356–369.

Jang, W., Youngentob, S.L. and **Schwob, J.E.** (2003) *Globose basal cells are required for reconstitution of olfactory epithelium after methyl bromide lesion.* J. Comp. Neurol., 460, 123–140.

Manglapus, G.L., Youngentob, S.L. and **Schwob, J.E.** (2004) *Expression patterns of basic helix–loop–helix transcription factors define subsets of olfactory progenitor cells.* J. Comp. Neurol., 479, 216–233.

Schwob, J.E., Youngentob, S.L. and **Mezza, R.C.** (1995) *Reconstitution of the rat olfactory epithelium after methyl bromide-induced lesion.* J. Comp. Neurol., 359, 15–37.

Schwob, J.E., Youngentob, S.L., Ring, G., Iwema, C.L. and **Mezza, R.C.** (1999) *Reinnervation of the rat olfactory bulb after methyl bromide-induced lesion: timing and extent of reinnervation.* J. Comp. Neurol., 412, 439–457.

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