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Invited Mini Review

Transcriptional regulatory network during development in the olfactory epithelium

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Regeneration, a process of reconstitution of the entire tissue, occurs throughout life in the olfactory epithelium (OE). Regeneration of OE consists of several stages: proliferation of progenitors, cell fate determination between neuronal and non-neuronal lineages, their differentiation and maturation. How the differentiated cell types that comprise the OE are regenerated, is one of the central questions in olfactory developmental neurobiology. The past decade has witnessed considerable progress regarding the regulation of transcription factors (TFs) involved in the remarkable regenerative potential of OE. Here, we review current state of knowledge of the transcriptional regulatory networks that are powerful modulators of the acquisition and maintenance of developmental stages during regeneration in the OE. Advance in our understanding of regeneration will not only shed light on the basic principles of adult plasticity of cell identity, but may also lead to new approaches for using stem cells and reprogramming after injury or degenerative neurological diseases. [BMB Reports 2015; 48(11): 599-608]

INTRODUCTION

Olfactory epithelium (OE) has representative characteristics of adult neurogenesis and regeneration. To capture the odor required for smell, olfactory receptor neurons (ORNs) are constantly exposed to the environment, including pathogens or other toxic substances, and thereby they are often confronted by degeneration. Therefore, ORNs have a shorter average life-span than that of other neurons. If the degenerated ORNs were not replenished, it would result in a total loss of the sense of smell. As chance would have it, ORNs and other cells in the OE are constantly renewed and replaced over their lifetime (1-4).

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The capacity of regeneration in the OE has been studied from experimental research in animals (5). Since the morphological reconstitution and functional restoration of OE after injury is relative simple, and the experimental in vivo studies are easy to apply, it offers a unique model system to study adult neurogenesis and regeneration (6-8). Adult neurogenesis occurs in two other areas in the central nervous system (CNS) beside OE: the subgranular zone (SGZ) which supplies new granule cells to the dentate gyrus of the hippocampus, and the subventricular zone (SVZ) which supplies new interneurons to the olfactory bulb (9-11). The OE is an area of particular interest as neurogenesis in the OE rarely exhibits tumor formation, although it follows a process similar to that of SGZ and SVZ in the CNS, presumably reflecting that tight regulation mechanisms might exist (12).

Over the past decade, efforts have been directed to identify the transcription factor (TFs) networks during adult neurogenesis and regeneration in the OE. Although there are a huge number of transcription factors (TFs) that are expressed in the OE, several studies have identified only a few TFs have expression profiles in cell proliferation, shifts in cell fate determination, and differentiation during regeneration. Once the regeneration process has started, the expression of TFs generally shares the pattern of embryonic and postnatal development (13-15). The progenitor cells undergo the same sequence during embryonic development, first actively proliferating, followed by the expression of Mash1, Neurog1, and NeuroD respectively, as they add new ORNs. This suggests that spatial and temporal expression of various TFs determines a cell's 'identity'; this fate selection is highly regulated within the network during regeneration of OE. Indeed, it has been proved for the first time that neurons have the capacity to reprogram their identity using nucleus of ORNs (16, 17). Hence, the demonstration of transcriptional regulatory network during regeneration in the OE provide new approaches in using stem cells to repair the adult CNS and sensory systems after injury or degeneration, and the reprogramming which originates from plasticity of the olfactory cell identity (18, 19).

This review describes the recent knowledge acquired about the transcriptional regulatory networks responsible for the proliferation, fate determination and differentiation of cells in the OE during adult neurogenesis and regeneration.

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Functional and structural features in the OE

The olfactory epithelium (OE) is a pseudostratified columnar neuroepithelium present on the nasal septum, and on a series of turbinates in the nasal cavity (Fig. 1A). Most studies about adult neurogenesis or regeneration of the OE have focused on the main olfactory epithelium (MOE) rather than the additional olfactory region such as the vomeronasal organ. There are three major layers in the OE: apical, intermediate, and basal layers. Supporting cells, immature and mature olfactory receptor neurons (ORNs), and horizontal and globose basal cells (HBCs and GBCs), reside in each layer respectively (Fig. 1B). Individual cell types can be identified based on a variety of physiologies (morphology, immune-reactivity and the location within the epithelium), in addition to the inference of their functions (20-23).

ORNs are bipolar, having a single dendrite that extends to the apical surface of the OE; the dendrite has a terminal knob covered with cilia. Upon odorant stimulation, the olfactory receptors in the cilia conduct initial events of olfactory signal transduction, and this signal is transmitted along the axons through the lamina cribosa to the olfactory bulb (24-26). ORNs are renewed throughout life at a regular rate, owing to naturally occurring regeneration (27-30). As they mature, ORNs shift from bottom to apical in the intermediate layers of the OE, reflecting neuronal age by position, with the expression of NCAM, NST and OMP (olfactory marker protein) (31-33).

Stretching from the epithelial surface to the basal lamina, supporting cells surround the ORNs, possessing glial functions in the OE (25, 34, 35). They structurally support and electrically isolate the ORNs (24, 36). They also have neuroprotective functions through the expression of cytochrome P450 isoform (37), as well as glutathione S-transferases (GSTs) (38) which are responsible for detoxification (39-41). They express several markers including SUS4, REEP6, Ezrin, K18 and Steel. Under normal conditions, the postnatal proliferation of supporting cells occurs very slowly, relative only to growth in the surface area of the OE; however, they comparatively appear more rapidly compared to the ORNs, after severe injury (42, 43).

Basal cells have been divided into two phenotypically distinct categories, the GBCs and HBCs, which are evident in the basal region. GBCs are round in shape and express GBC-1, GBC-3, and GBC-5 as markers (44, 45). In normal conditions, they are mitotically active, dividing once per day, with the expression of proneural genes. Some of them are currently considered to be neuronal progenitor cells. HBCs are flat in shape and make direct contact with the basal lamina above the underlying lamina propria. ICAM1 (CD54) and keratin-K5 are representative markers. HBCs serve as reserve pools of long-lived progenitors since they are relatively quiescent, dividing once every 60 days under normal regeneration of ORNs, but proliferate more actively during injury-induced regeneration (3, 46-49). Thus, the basal cells continuously generate ORNs and other types of cells throughout life as well as after damage of the OE (15, 50). Since both basal cells have the capacity as progenitors, their relationship remains elusive as to whether HBCs give rise to GBCs, or vice versa. Recently, it was established that GBCs committed to the ORN lineages descended from keratin K5-expressing HBCs (48). This study puts forward evidence to support the hypothesis that the HBCs are the multipotent progenitors that give rise to all cell types in the OE, including GBCs, in the controversy surrounding 'the' real olfactory stem cell. Detailed roles and mechanisms of basal cells in the OE contributing to the continuous production of new ORNs, and other cells of the OE, will be described later in the review.

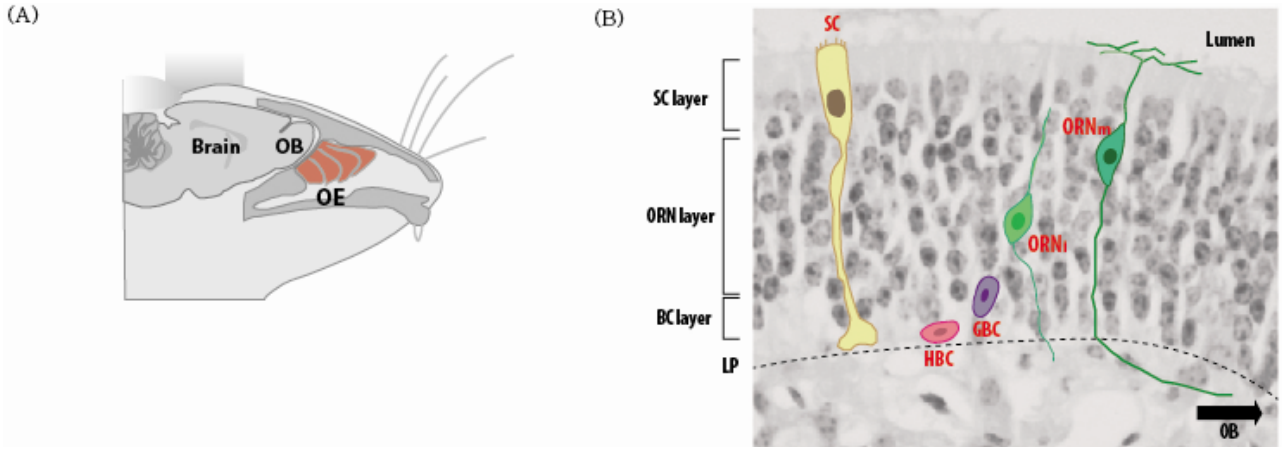

Fig. 1. Structure of olfactory epithelium (OE). (A) Sagittal plane of the rodent nose elucidating the location of the olfactory epithelium (red). (B) The olfactory epithelium is composed of several cell types from apical to basal layers: supporting cells (SCs), immature and mature olfactory receptor neurons (ORNs), and globose and horizontal basal cells (GBCs and HBCs). LP: lamina propria.

Adult neurogenesis and regeneration in the OE

The OE retains active processes for supplying new ORNs throughout life; it also has the capacity to regenerate in response to many types of damage. Under normal conditions, ORNs degenerate and die constantly, being replaced by the proliferation and differentiation of basal cells every few days (51). Severing axons by axonal dissection or olfactory bulbectomy (ablation of olfactory bulb) triggers apoptosis in ORNs, and proliferation of basal cells within a few days (52). Complete restoration of the OE takes almost 4 weeks, and new ORNs target their axons and recover their functional synapses in the OB. Upon injury or exposure to chemical toxins such as MeBr or zinc sulfate, which cause massive degeneration of ORNs, supporting cells and GBCs, the entire OE takes several months to be restored to its prelesion state (43, 53, 54).

During adult neurogenesis and regeneration, cell proliferation and cell fate determination for differentiation is preceded by developmental interactions among the comprised cells in the OE. The identification of cells involved in adult neurogenesis and regeneration have been studied. GBCs and HBCs have emerged as candidate stem cells of the OE for years. It is considered that GBCs are neuronal progenitors committed to the ORN lineage passing through transit amplifying cells or late immediate neuronal precursors (INPs) in the OE (13, 51, 55, 56). However, several studies suggest that they also act as multipotent progenitors to generate all of the other types of the olfactory cells (15, 45, 57, 58). Following mild injury, in which the damage is limited to ORNs (such as olfactory bulbectomy or olfactory nerve transection), GBCs are likely to increase their proliferation and rapidly reconstitute the lost cell types (59). HBCs act like realistic olfactory stem cells with the hallmark of well-characterized adult stem cells, having very slow proliferation under normal regeneration, which is sufficient to maintain the GBC population. After extensive damage, they can give rise to all of the different cell types in the OE by cre-lox lineage tracing studies (48, 49, 60, 61).

In the neuronal lineage of the OE, GBCs consisting of early transit-amplifying cells, and INPs which express proneural genes such as Mash1 and Ngn1, are involved in the regular renewal of ORNs after injury (Fig. 2A) (56). INPs give rise to daughter cells that exit the cell cycle and differentiate into immature ORNs, move to the apical region in the intermediate layers (1, 55). Differentiation of these cells into mature ORNs involves the expansion of a dendrite and axon at the opposite poles of the neurons. Terminal differentiation of mature ORNs climaxes with establishment of synapses at glomeruli in the olfactory bulb, targeting on a particular region from sensory neurons expressing the same odorant receptor (62-66). In the non-neuronal lineage of the OE, supporting cells are first driven from the basal cells after injury with expression of Hes1, which is known to suppress transcription factor (TF) of proneural genes (Fig. 2B) (57, 67, 68). Taken together, it suggests that the regeneration of ORNs and other cell types in the OE may keep pace with the increased loss of these cell population

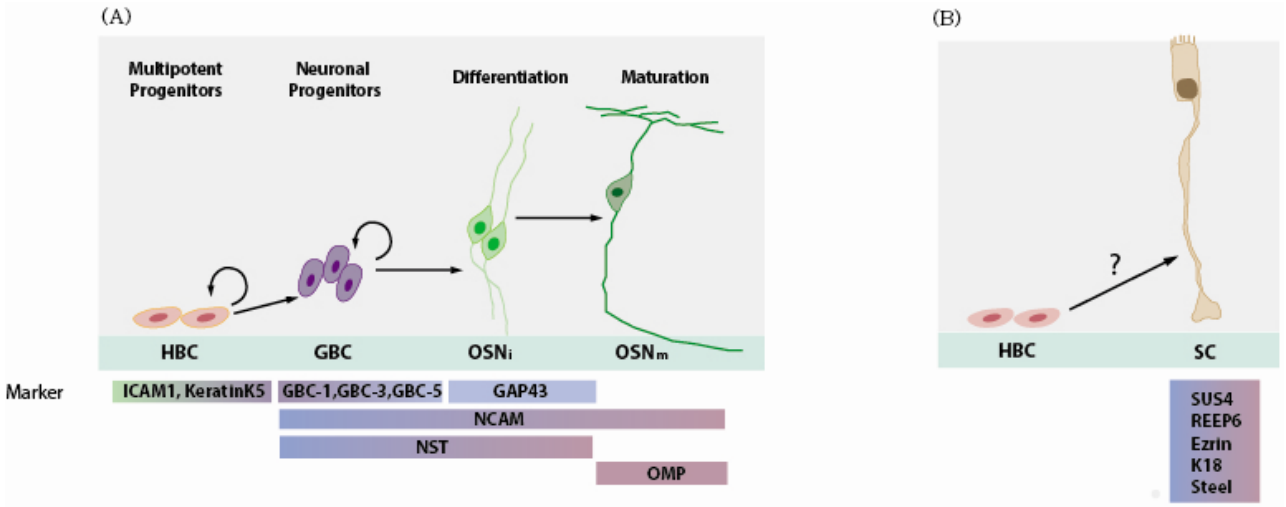

Fig. 2. Process of regeneration in the OE. The OE retains active processes for supplying new ORNs and supporting cells during regeneration. These cell types can be distinguished by several markers. (A) During ongoing ORNs generation, HBCs and act as olfactory stem cells for other cells in the OE. After neuronal cell fate determination of GBCs, they differentiate and maturate into ORNs. (B) In the non-neuronal lineage of the OE, supporting cells are generated from HBCs, but detailed process is under investigation. GBCt: transit amplifying globose basal cell, GBCinp: immediate neuronal precursor globose basal cell, ORNi: immature olfactory receptor neuron, ORNm: mature olfactory receptor neuron, ICAM1: Intercellular Adhesion Molecule 1, GAP43: 43kDa growth associated protein, NCAM: neural cell adhesion molecule, NST: neuron specific tubulin, OMP: olfactory marker protein, SUS4: sucrose synthase 4, Reep6: Receptor Accessory Protein 6, K18: keratin 18.

or changes of cell type compositions, accompanied by aging or environmental factors.

Transcriptional network during adult neurogenesis and regeneration in the OE

The network of transcription factors (TFs) by which adult neurogenesis and regeneration is regulated in the OE remains unclear. Many have yet to be clearly defined, perhaps because the TF expression patterns that manage the initial development of embryos has been established, but not in adult neurogenesis and regeneration. It has been investigated by up- vs. down- experiments to identify master regulators to generate ORNs and other cell types in the OE from basal cells, using the regeneration model system. The determination of cell types in the OE shares some common TF expression patterns that are similar to those expressed at the embryonic or postnatal stages. We will highlight a few master TFs in the adult neurogenesis and regeneration of OE.

Maintenance (Proliferation) of multipotent progenitors: The TFs Sox2 and Pax6 are required for maintenance of multipotent progenitor cells in the OE, similar to the other regions of the nervous system. In the embryonic development of OE, they often function together at a very early stage in the nasal placode for initial formation of olfactory sensory epithelium (69). Sox2 (SRY-box containing gene 2) is a member of the Sox family, which contains the high-mobility group (HMG); it is one of the key factors in regulating the embryonic stem cell self-renewal and inducing somatic cells to become pluripotent stem cells (70-72). In the nervous system, Sox is a persistent marker for multipotent progenitor/stem cells isolated from the embryonic CNS; also, using the Sox2-GFP mouse line, it has been shown that the adult CNS strongly expresses Sox at the region where adult neurogenesis occurs (71, 73). Sox2 is expressed in both the basal and apical layers in the OE, corresponding with the location of proliferating cells at a very early developmental stage. Blocking the neuronal lineage in the OE, Sox2 expression expands to most of the cells beyond its initial expression region (4, 69). Pax6 (Paired box 6) is a member of paired box family and contains paired domain and partial or complete homeo box domain. It regulates the multipotent progenitor cells in diverse systems, including CNS (74). Studies show that Pax6 is involved in olfactory development, since nose formation failed in the Pax6 mutant animals (75). It is also expressed in adult OE, especially cells of the non-neuronal lineages, including HBCs and supporting cells (76). The involvement of Sox2 and Pax6 in the development of OE was identified from genetic studies, where Sox2 and Pax6 were expressed by a variety of cell types in the normal adult and MeBr lesioned-regenerating OE. Both TFs are expressed in supporting cells, multipotent GBCs, Mash1+ GBCs (commonly committed to the neuronal lineage), and HBCs (43, 76, 77). Sox2 and Pax6 may play multiple and complex roles in the regulation of olfactory neurogenesis as well as for the maintenance of multipotency (Table 1, Fig. 3).

Recent studies implicated that p63 also has an important role in maintenance of multipoteny in the OE. p63 is a member of the p53 tumor suppressor gene family known to maintain self-renewal or cell survival of stem cells in other stratified epithelia (78, 79). Using p63 null mutation germline, it was confirmed that p63 is required for the generation of HBCs dur-

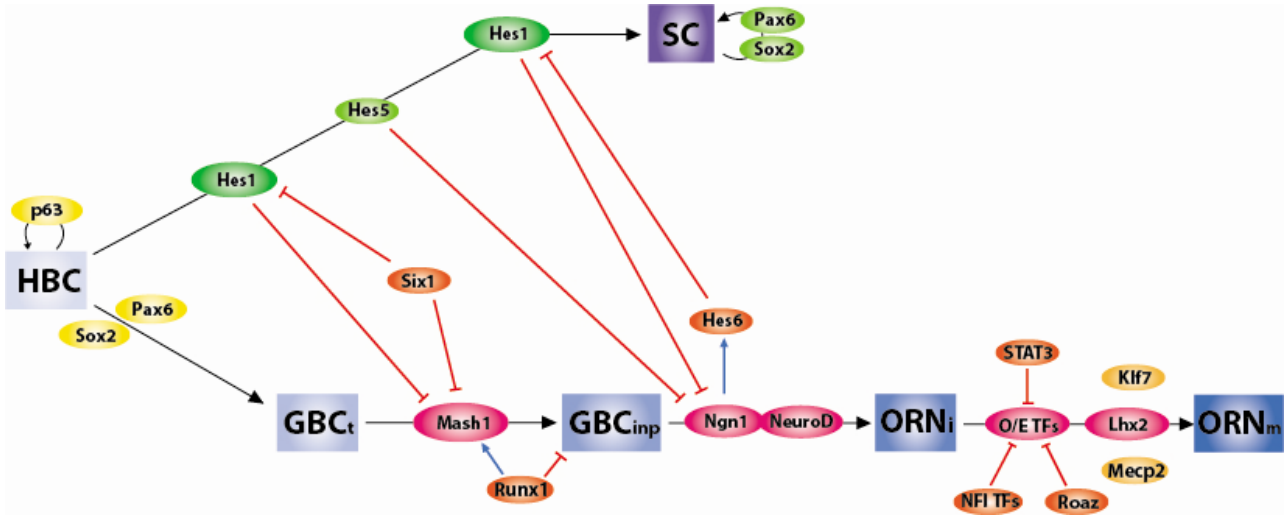

Fig. 3. Scheme of transcriptional regulatory network during regeneration in the OE. Sox2, Pax6 and p63 regulate proliferation and differentiation of multipotent progenitor cells. Two representative bHLH TFs, Mash1 and Hes1 might have role in the determination of cell fate between neuronal and non-neuronal lineages. And their multiple component of the downstream get involved in differentiation and final maturation of each cell fate. GBC_t: transit amplifying globose basal cell, GBC_{inp}: immediate neuronal precursor globose basal cell, ORN_i: immature olfactory receptor neuron, ORN_m: mature olfactory receptor neuron.

ing embryogenesis (80). p63 contributes to maintain HBCs self-renewal and is not involved in their differentiation, as HBCs can give rise to differentiated cells of the OE during injury-induced regeneration in the conditional p63 knockout (61). Although the possible interactions among p63, Sox2 and Pax6, which are relevant to multipotency in adult OE have not been clearly defined, Sox2 and Mash1 seems to be affected by conditional p63 knockout (61) (Table 1, Fig. 3).

The TF regulation of multipotent progenitor cells has led to the proposal that they form a transcriptional regulatory network for keeping their balance between proliferation and differentiation in the adult OE.

Neurogenesis: During regeneration, progenitors undergo several processes to make various cell types which comprise the OE; these include cell fate determination between neuronal and non-neuronal lineages, their differentiation and maturation. In the OE, Mash1 and Hes1 function as a molecular switch that determines the cell fate of the two cell lineages, the ORNs and non-neuronal cells, especially the supporting cells (Fig. 3). Two representative bHLH (basic helix-loop-helix) TFs act as transcriptional activators or repressors to each other, during embryonic neuronal development of OE (68). Targeted knockout animals, overexpression studies, and the changes in spatial or temporal expression of multiple components downstream of Mash1 and Hes1 following injury, emphasize its importance in regulating olfactory epithelial cell fate. These transcriptional regulations that govern embryonic and adult neurogenesis/regeneration overlap each other, but are not identical.

Mash1 (Mammalian Achaete Scute Homolog 1) is a member of the bHLH family; it plays a role in neuronal commitment and differentiation at the selected point in the olfactory system (13, 14, 81-83). Mash1 is expressed at most layers in the developing OE, especially in transit amplifying progenitor cells, but not in differentiated ORNs (13, 56, 84). The generation of ORNs is blocked almost completely by depletion of Mash1 during the embryonic stage. Loss of Mash1 results in the fail-

Table 1. Changes of TFs during regeneration in the OE

Stage	TFs	Family	Localization	Age	Network	Ref
Maintenance of multipotent progenitors	Sox2	Sox	Most layers	E	(+) Pax6	(77)
	Pax6	Paired	Most layers	E, P, A	(+) Sox2	(77)
	P63	P53 tumor suppressor gene	B	P, A		(61)
Neuronal cell fate determination	Mash1	bHLH	Most layers	E, P, A	(−) Hes1 Six1 (+) Runx1 Wt1 (∼) Pax6 Sox2	(13-15, 56, 115-118)
	Six1	HD-Six	B, A	E, A		(90. 119)
	Lhx2	LIM	B, I	E, A	(+) NeuroD (∼) Mash1 Ngn1	(14, 101, 120, 121)
ORN differentiation	Ngn1	bHLH	B	E, A	(+) Mash1 (−) Hes1 Hes5	(13-15)
	NeuroD	bHLH	B	E, P, A	(+) Ngn1 Runx1	(112)
	Hes6	bHLH	B	E, P, A	(+) Ngn1	(119, 122)
	Runx1	Runx	B	E, P		(84)
ORN maturation	O/E TFs	O/E (Olf/EBF)	B, I	E, P, A	(+) NeuroD (−) NF I TFs STAT3 Roaz	(14, 76, 98) (94-96)
	STAT3	STAT	?	P, A		(99)
	NF I TFs	NF I	Most layers	E, P		(98)
	Roaz	Zn finger	B, I	A		(102)
	Mecp2	MBD	Most layers	E, P, A		(103. 106)
	Klf7	Zn finger	?	E, P		(104, 105, 107)
Non-neuronal differentiation	Hes1	bHLH	A	E, A	(+) Hes5 (−) Hes6	(14. 108)
	Hes5	bHLH	B	E, A	(+) Hes1	(14. 108)
	Sox2	Sox	Most layers	E, P, A		(77)
	Pax6	Paired	Most layers	E, P, A		(77)

Localization; B: basal, I: intermediate, A: apical, Age; E: embryonic, P: postnatal, A: adult), (+): promotion, (−): inhibition, (∼): unrelatedness (modified from (116)).

ure of the sequential expression of Ngn1 (Neurogenin1) and NeuroD, which culminate in the production of ORNs (4, 13, 62, 85, 86). Thus, Mash1 could be crucial for progression to a neuronal fate at early stage of ORN development (Table 1, Fig. 3).

The effects of Mash1 expression on adult neurogenesis and regeneration has also been studied extensively. In the developing epithelium after olfactory bulbectomy or exposure to MeBr, Mash1 and proneuronal bHLH TFs, Ngn1 and NeuroD are sequentially expressed in the similar expression pattern during embryonic development of the OE. The epistatic relationship and the timing of their expression implicates that Ngn1 and NeuroD act downstream of Mash1, and have features of INPs. Ngn1 and NeuroD are expressed in cells of the GBC layer, and in cells above Mash1+ GBCs. In Ngn1 (Neurogenin1) null mutant OE, multipotent progenitors are generated, but their initial differentiation to ORNs is blocked. NeuroD follows after the Ngn1 expression during terminal-differentiation of ORNs under regeneration (14, 15, 77, 87-89) (Table 1, Fig. 3).

Multiple TFs have been suggested or shown to act along with with Mash1, Ngn1 and NeuroD1 to continue neurogenesis in the OE. Six1, a member of the HD-Six family, seems to regulate the determination of cell fate in the developing OE. Its expression increases simultaneous with the Ngn1 expression after olfactory bulbectomy (88). It was observed that the expression of Mash1 and Hes1 increases in Six1 KO mice (90). Runx1, a member of the Runx family, is important in preventing premature differentiation of multipotent neuronal progenitors in the OE, and Runx1 expression was seen to increase after olfactory bulbectomy. The Runx1-deficient mice exhibit a significant decrease in the number of NeuroD+ cells, but have an unaltered expression of Mash1 (84, 88). Hes6 seems to promote neuronal differentiation in the OE, being downstream of Ngn1, perhaps by suppressing Hes1 (89). These studies reveal that three bHLH TFs and other networking TFs function as the determining factors or differentiation factors, depending on the timing of their expression in neuronal progenitors in the OE (Table 1, Fig. 3).

There are many other TFs which are involved in ORN maturation. In particular, O/E TFs (Olf/EBF TFs) play a key role in the stage that enhances the expression of OMP, Golf, OcNc and, ACIII binding to their promoter regions (91-95). O/E TFs deficient mice fail to format and innervate the synapse with glomerulus in the OE (96). As OMP expression is also detected in the OE/TFs deficient mice, it is believed that other numerous TFs also contribute to ORN maturation and functions (96, 97). Nuclear factor I (NF I) TFs and STAT3 bind to OMP promoter and negatively regulate the expression of genes involved in terminal differentiation (98, 99). It is assumed that NF I TFs interfere with O/E TFs, as the mutation of NF I binding sites in the OMP promoter region results in OMP activation by O/E TFs (98, 100). Lhx2 (LIM-homeodomain 2) gets involved in the terminal differentiation of ORNs, since Lhx2 conditional inactivation in ORNs cannot innervate their target in the olfactory bulb (101). Although Roaz (a rat C2H2 zinc finger protein), Mecp2 (methyl CpG binding protein 2), and Klf7 (Kruppel like TF 7) were detected in the mature ORNs, and their expression changed at the maturation stage after olfactory bulbectomy or embryonic development, the mechanisms which mediate the maturation process remains to be determined (14, 76, 102-107) (Table 1, Fig. 3).

Non-neuronal differentiation: Hes1 (mammalian homolog of Drosophila Hairy and Enhancer of split 1) (108), is a member of the bHLH family, and several studies have shown that Hes1 in particular has important roles in non-neuronal differentiation in the OE (Fig. 3) (109-113). Following Hes1 null mutant mice study, it was found that initially, the Hes1 functions in restricting a region of proneuronal gene expression at the onset of neurogenesis, and subsequently inhibits the recruitment of neuronal progenitors in the embryonic OE. Mash1 interacts with Hes1 for these two functions (108). Hes1 is expressed by the supporting cells of apical layers in the normal adult OE or after olfactory bulbectomy (15). After MeBr treatment, Hes1 is expressed in the basal cells, Hes1+ basal cells are displaced to the apical end as regeneration progresses, and finally differentiates into the supporting cells (13, 108). Hes1 expression seems to mark the commitment to supporting cell fate with suppression of Mash1 in neuronal progenitors. There are also transcriptional interactions between Hes1 and other TFs to proceed to non-neuronal differentiation. Hes5 functions as a repressor in neurogenesis synergized with Hes1, as it regulates the expression of Ngn1 exclusively (108, 114). The Hes1 and Mash1 seem to have a reciprocal relationship during regeneration. Mash1 reappears after Hes1 expression, during regeneration following MeBr lesions. Loss of Mash1 induces the elimination of Hes1 expression, while loss of Hes1 allows an extension of the Mash1 expression and the production of neurons in the developing OE (13, 108) (Table 1, Fig. 3).

These studies reveal that making a choice between ORNs and supporting cells is followed by expression of two types of TFs: transcriptional activators that drive neuronal lineage, including Mash1, Ngn1 and NeuroD, and transcriptional repressors of neuronal differentiation that belong to the Hes group (15). Further insight into the network of these molecular signals which controls the choice between making ORNs vs. non-neuronal cells in the OE, should be gleaned from future studies focusing on the mechanisms and interacting partners.

CONCLUSION

The past decade has witnessed a decrease in stem cell transplantation for injuries related to the nervous system, and the introduction of new approaches of regenerative medicines. These approaches are now focused on the regulatory mechanisms of ongoing cell replacement which facilitates robust regeneration in the OE, which otherwise do not appear in the retina or cochleae. Numerous TFs are expressed in each cell

type of OE, along with spatial and temporal differences in the features of the transcriptional regulatory networks that characterize them during regeneration. Deciphering these networks is likely to provide new insights into the regulation of cell identity in the OE. It is also evident that TFs are powerful modulators of development as they can induce the transition between different cell states. There are many aspects of the regulation of OE regeneration that still remain unknown. But one can imagine a scheme in which these pathways could be targeted by gene therapy to maintain multipotent progenitors, or initiate a process of regulated reprogramming for facilitating neurogenesis in regions of the adult nervous system where regeneration is limited or absent. Indeed, viral expression of Atoh1 has already been shown to regenerate some hair cells from damaged cochlea in the mammalian inner ear (115). Understanding the TF mechanisms regulating adult neurogenesis and regeneration will enable the development for cell replacement therapy, using either endogenous progenitors or reprogramming cells from different sources, and possibly create novel ones.

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