

REVIEW

Translational potential of olfactory mucosa for the study of neuropsychiatric illness

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The olfactory mucosa (OM) is a unique source of regenerative neural tissue that is readily obtainable from living human subjects and thus affords opportunities for the study of psychiatric illnesses. OM tissues can be used, either as *ex vivo* OM tissue or *in vitro* OM-derived neural cells, to explore parameters that have been difficult to assess in the brain of living individuals with psychiatric illness. As OM tissues are distinct from brain tissues, an understanding of the neurobiology of the OM is needed to relate findings in these tissues to those of the brain as well as to design and interpret *ex vivo* or *in vitro* OM studies. To that end, we discuss the molecular, cellular and functional characteristics of cell types within the olfactory mucosa, describe the organization of the OM and highlight its role in the olfactory neurocircuitry. In addition, we discuss various approaches to *in vitro* culture of OM-derived cells and their characterization, focusing on the extent to which they reflect the *in vivo* neurobiology of the OM. Finally, we review studies of *ex vivo* OM tissues and *in vitro* OM-derived cells from individuals with psychiatric, neurodegenerative and neurodevelopmental disorders. In particular, we discuss the concordance of this work with postmortem brain studies and highlight possible future approaches, which may offer distinct strengths in comparison to *in vitro* paradigms based on genomic reprogramming.

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INTRODUCTION

A critical component of neuropsychiatric research is the delineation of neurobiological abnormalities in patients' brains. Although decades of postmortem studies have yielded vital insights, the lack of access to living patients' brain tissues has long been a major hurdle in the field. Recently, several *in vitro* paradigms have emerged, such as induced pluripotent stem cell (iPSC)¹ and induced neuronal² cell technologies, which offer unique and unprecedented opportunities to reprogram patients' cells into developing neurons and glial cells.

This review focuses on another paradigm with a similar purpose and with distinct strengths; the olfactory mucosa (OM) tissue approach. The OM harbors neurons and glial cells residing in the nasal cavity and is readily accessible via biopsy. Neural tissues without genomic reprogramming can be captured via olfactory biopsy. OM tissues offer *ex vivo* and *in vitro* neuronal cells that may more closely reflect *in vivo* neural characteristics of the donors. OM cells also have regenerative potential, which permits them to propagate *in vitro*, yielding neuronal or glial cells in sufficient quantity for various assays.

Another unique advantage of this paradigm arises from the fact that the OM is the peripheral component of the olfactory circuitry, connecting olfactory sensory neurons (OSNs) to the olfactory bulb, the neurons of which then connect to the olfactory cortex. As such, neurobiological alterations in patients' limbic regions may affect or be reflected in the olfactory circuitry, including neural cells in the OM. Indeed, Alzheimer's disease,^{3,4} schizophrenia^{5–9} and mood and anxiety disorders (review¹⁰) are associated with

olfactory dysfunction, and OM derived from these patients exhibit cellular and molecular alterations.^{11–20}

Recently, a growing number of groups have examined OM biopsies in these disorders^{11,12,17,21–23} and others in which olfactory dysfunction is yet to be characterized as a key phenotype.^{14,24–26} An important question is to what degree and/or in what ways these OM-derived cells reflect the pathophysiologic mechanisms in patients' brains. Given that the OM is a specialized neural tissue containing a regenerative neuroepithelium whose identity is distinct from brain cells, alterations in the OM cells can best be appreciated in the context of the neurobiological characteristics specific to the OM tissue. The goal of this paper, therefore, is to consider how the OM paradigm can contribute to pathophysiologic understanding of neuropsychiatric illnesses by reviewing the neurobiological context in which these OM cells arise *in vivo* and *in vitro*.

CYTOARCHITECTURE AND MOLECULAR CHARACTERISTICS OF THE OM

A distinctive characteristic of the OM is that it contains a regenerative neuroepithelium. In this neural tissue, cells continually slough off and are replenished by newborn cells including neurons and other cell types. Multipotent cells of the OM must continuously produce replacement OSNs and other cell types in the adult mammalian OM.^{27,28} This continual process of renewal from multipotent cells to differentiated neurons offers a unique

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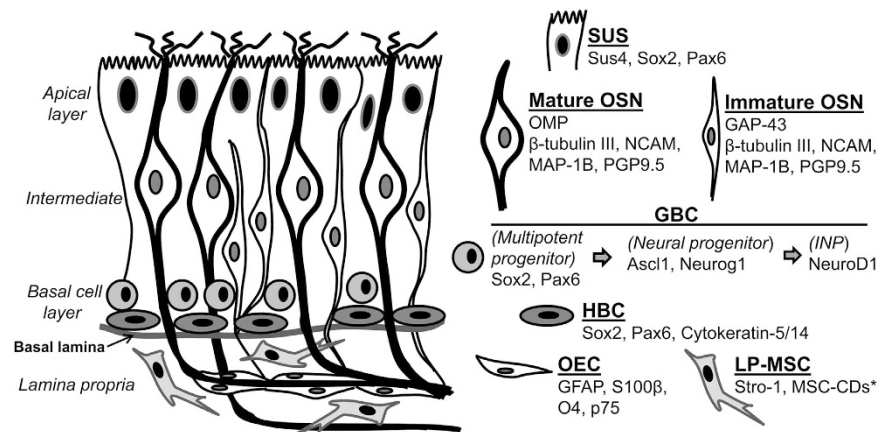


Figure 1. Cytoarchitecture and molecular characteristics of the olfactory mucosa (OM). The multilaminar organization of the rodent OM is depicted on the left, and core molecular markers that define and distinguish mammalian OM cell types *in vivo* are listed on the right. Most of the studies identifying these markers have been reviewed elsewhere.^{32–36} *Markers that distinguish lamina propria mesenchymal stem cells (LP-MSCs) have not been studied to the extent of markers for other OM cell types, but see Tome *et al.*,³⁷ Lindsay *et al.*,³⁸ and Delorme *et al.*³⁹ for MSC-specific cluster of differentiation (CD) expression in these cells. GBC, globose basal cell; HBC, horizontal basal cell; INP, intermediate neural progenitor; OEC, olfactory ensheathing cell; OSN, olfactory sensory neuron; SUS, sustentacular cell.

opportunity to capture the processes of neurodevelopment in human studies.

Although the OM is located external to the brain, OSN axons extend through the lamina propria (LP) and then the cribriform plate to the brain where they synapse onto neurons of the olfactory bulb. These olfactory bulb neurons then project to primary olfactory regions, including the piriform and entorhinal cortices and the amygdala.²⁹ Cells within the projection regions then synapse onto cells in other neuropsychiatric disease-related regions, including the hippocampus, prefrontal cortex and hypothalamus, thus enabling olfactory information to influence emotional, cognitive and visceral functions.^{10,29}

The current knowledge of the cytoarchitecture and molecular profiles of the OM stems largely from rodent studies. The human olfactory neuroepithelium (ON) is thought to recapitulate histologic and molecular characteristics of the rodent counterpart, though the precise degree of similarity remains under investigation. The ON exists as a pseudostratified epithelium in adult mammals, comprising three primary layers; a basal layer, an intermediate region and an apical layer (as depicted in Figure 1). The apical layer is exposed to the nasal cavity and contains glial-like supporting cells called sustentacular cells. The intermediate region contains layers of immature and mature OSNs and exhibits a distinct laminar organization in the rodent ON (Figure 1), with the immature OSN layer residing closer to the basal lamina. In the human ON, however, immature and mature OSNs are more dispersed throughout, suggesting a less pronounced laminar organization of OSNs than observed in the rodent ON.^{30,31}

Deep to the intermediate region lies the basal layer that contains globose and horizontal basal cells (GBCs and HBCs, respectively), and is separated from the underlying LP by the basal lamina. As shown in the mouse ON, GBCs and HBCs are the stem cells that initiate the OSN lineage by proliferating into transit-amplifying progenitors that give rise to immediate neuronal precursors (INPs), which differentiate into immature and ultimately mature OSNs.³² Several rodent studies have delineated the expression of transcription factors in ON basal cells to define the lineage of ON cells.^{33,34} These defining factors and associated cell types are listed in Figure 1. Depending upon the niche, both HBCs and GBCs comprise stem cell populations in the rodent ON that can be both neuro-competent and multipotent.^{28,40}

In the mouse ON, the basal layer exhibits a distinct organization. HBCs form a single layer next to the basal lamina, exhibit a flattened morphology and express cytokeratin-5 and -14, whereas

GBCs exhibit a round morphology, do not express cytokeratins and reside in a single layer superficial to the HBCs (Figure 1).^{41,42} Similarities between rodent and human ON basal cells have been observed,^{30,31,43} yet the laminar organization and morphologies of the different basal cell types (flattened versus rounded) appear to be less clearly defined in human ON.^{30,31} In addition, molecular expression patterns of these basal cells may differ between species, as robust expression of the low affinity nerve growth factor p75NGFR has been observed in first- and second-layer basal cells in human ON, but not in adult rodent ON.^{13,30,44,45} These basal cells, whether analogous to rodent HBCs, GBCs or both, are thought to comprise the presumptive neural precursor population in human ON.

Juxtaposed to the basal cell layer, separated by the basal lamina, is the LP. Rodent studies have shown this to be a neural crest-derived ectomesenchymal tissue, which contains multipotent mesenchymal stem cells (LP-MSCs), olfactory ensheathing cells (OECs), axons originating from the OSNs and the acynous of Bowman glands.^{37,46,47} Studies of LP-MSCs in rodents and humans have shown that these cells are able to generate multiple cell phenotypes, including, but not limited to, OECs and neural lineages, both during development and in response to regenerative cues.^{35,37,38,46,47,48} The glial potential of OECs has been extensively studied in the context of spinal cord transplantation and has been reviewed elsewhere.^{49,50} OECs represent a highly specialized, likely heterogeneous glial cell population that share morphological and neurochemical features with Schwann cells and astrocytes, although they differ from both. Their neurochemical composition highlights their functional heterogeneity, as they express sets of proteins shared with astrocytes (for example, glial fibrillary acidic protein (GFAP) and s100β), oligodendrocytes and oligodendrocyte precursors (O4, NG2), CNS radial glia (basic lipid binding protein, BLBP), as well as developmentally important proteins CD 44, β1 integrin, P200 and Notch 3.³⁶

USE OF EX VIVO OLFACTORY TISSUE IN THE STUDY OF NEUROPSYCHIATRIC AND NEURODEVELOPMENTAL DISEASES

As a regenerative neuroepithelium containing a range of morphologically and molecularly distinct neuronal and glial cells, the *ex vivo* ON represents a useful tool for examining cell type-specific biological changes in neuropsychiatric illness. Compared with all other *in vitro* neuronal models or blood cell studies, this cell type-specific resolution is a unique feature of this paradigm.

As such, the ON has been utilized as a platform for histologic assessment, investigation of intracellular signaling and gene expression profiling.

Using a histologic approach to study neuronal differentiation, Arnold *et al.*¹³ conducted immunohistochemical assessment of the ON using markers for specific stages of neuronal differentiation in *ex vivo* tissue from schizophrenic patients and controls. In this study, basal cells, immature and mature neurons were marked with antibodies for p75NGFR, GAP-43 and OMP, and densities of immunoreactivity for these markers were used as indices for specific stages of differentiation. Compared with controls, patients exhibited decreased density of p75-labeled basal cells, a higher density of GAP-43-labeled immature OSNs and an increased ratio of immature to OMP-labeled mature OSNs. Together these findings led to the postulate that neuronal lineage may be disrupted in the ON and by extension in the CNS of patients with schizophrenia.¹³

In a similar paradigm, Pantazopoulos *et al.*²⁰ examined chondroitin sulfate proteoglycans, prominent components of the extracellular matrix, in *ex vivo* ON from schizophrenic patients compared with controls. These extracellular matrix proteins are critical for cellular differentiation and migration, and are postulated as a possible mechanism for the altered neuronal lineage as observed in by Arnold *et al.* In that study, the decreased chondroitin sulfate proteoglycan density reported in mature OSNs in schizophrenia is consistent with previous findings of reduced proteoglycans in multiple brain regions of postmortem brains of schizophrenic patients.^{20,51} Thus, some of the neurobiological characteristics observed in the ON can be extrapolated to those of the brain.

Histologic changes in the ON that are specific to particular neuropsychiatric illnesses may hold promise as potential biomarkers. In the postmortem ON of patients with Alzheimer disease, Arnold *et al.* found higher frequency and abundance of amyloid- β and paired helical filament-tau pathologies in ON derived from Alzheimer patients compared with controls,¹¹ essentially mirroring previous observations in postmortem brains of patients (reviewed in Hardy and Selkoe⁵²). If these and other findings described above are extended to ON biopsy tissues of living patients, and are correlated with clinical severity or diagnostic subtypes, they could serve as cellular biomarkers of psychiatric illnesses.

The ON can be utilized for gene expression profiling of neural cells with cell type-specific resolution. McCurdy *et al.*¹⁴ used microarray analyses in *ex vivo* tissues to demonstrate altered expression of genes relating to cell cycle and neurogenesis in schizophrenic patients, findings that may be consistent with neurodevelopmental dysregulation. More recently, Mor *et al.*²¹ used laser capture microdissection in *ex vivo* tissues to isolate mature OSNs, and observed increased miRNA expression of MiR-382 in schizophrenic patients versus controls. Notably, similar dysregulation in MiR-382 has been observed in the postmortem dorsolateral prefrontal cortex,⁵³ again supporting the notion that certain aspects of brain pathology may be represented in the ON.

Ex vivo ON tissues can also be examined for their electrophysiological properties. OSNs can be dissociated from biopsy tissues, readily identified by their morphology and examined using calcium imaging, voltage sensitive dye imaging and other electrophysiological measures. Indeed, Hahn *et al.*¹⁹ assessed odorant induced calcium signaling in OSNs derived from medication-free bipolar disorder patients and their matched controls, and demonstrated decreased intracellular calcium signaling in bipolar disorder patients. Given that dysregulated calcium signaling has been consistently reported in bipolar disorder patients in peripheral blood and brain,^{54–56} ON derived from bipolar patients may be useful for furthering understanding of the molecular pathologies of the disorder.

Ex vivo ON tissues also provide a unique paradigm for the study of neurodevelopmental disorders, given the lifelong regeneration

of ON and the presence of OSNs at all the stages of development. One such developmental disorder where *ex vivo* ON has been studied is Rett syndrome, which results in profound intellectual disability and has been associated with deficits in neuronal maturation. Ronnett *et al.*²⁶ reported fewer mature OSNs, increased immature OSNs and abnormal morphology of mature OSNs in Rett syndrome patients of various ages compared with age-matched controls. These observations support the notion that ON is a viable tool for studying neurodevelopmental aspects of diseases characterized by abnormal neuronal development.

IN VITRO USE OF CULTURED OM-DERIVED CELLS IN THE STUDY OF NEUROPSYCHIATRIC ILLNESSES

A second approach to the use of the OM in psychiatric research, which complements the study of *ex vivo* OM tissues, is the *in vitro* culture of OM-derived cells. Due to the regenerative nature of the OM, OM-derived cells can be readily propagated and are increasingly yielding insights into the cellular and molecular underpinnings of neuropsychiatric illness.

Properties of adult human OM-derived cells *in vitro*

When developing *in vitro* models for neuropsychiatric illnesses, it is critical to consider the specific characteristics of cell types arising in culture, which are determined by the tissue compartment from which they originate and conditions in which they are grown. In diverse variations of these factors, multiple approaches to the culture of OM-derived cells have been developed including the organotypic explants, dissociated cultures and neurospheres (see Box 1 for specifics of culture paradigms). Common to all these paradigms is that they seek to utilize regenerative characteristics of the ON and its underlying LP as neural tissues, within which progenitors differentiate into neurons, sustentacular cells and OECs.^{57–59}

The experimental utility of cultured OM cells as a model for neuropsychiatric illness is, in part, related to the extent to which cultured cells recapitulate the characteristics of *in vivo* OM cells or of donors' neurobiological makeup. To define neuronal precursors, immature neurons or OSNs, a rapidly increasing database on the expression of molecular markers in cell types of each differentiation stage can be used as a guide. As described in Figure 1, human and rodent ON cells at different stages of neuronal differentiation *in vivo* are characterized by expression of molecular markers such as Cytokeratin-5, Sox2, Pax6, Ascl1, Neurog1, NeuroD1, Gap43 and OMP. Neuronal precursors can be defined as proliferative cells whose progeny ultimately become neuronal cells. Immature neurons and OSNs do not share this proliferative capacity, and OSNs are further defined functionally by their capacity to respond to odorants.^{70,71}

Numerous markers have been used to characterize OM-derived cells *in vitro* (Figure 2). While some markers, such as NeuroD1 and NCAM, are selectively expressed in neuronal cells at varying stages of differentiation *in vivo*, the selectivity or appropriateness of other markers is less clear. For example, β -tubulin III and vimentin are expressed not only in olfactory neurons *in vivo*, but also in other cell types,^{72,73} thus limiting their utility as sole markers for neurons *in vitro* despite their widespread expression. While nestin has been recognized as a neural stem cell/progenitor marker in the mammalian brain, its expression in adult human ON *in vivo* has been demonstrated exclusively in sustentacular cells as opposed to basal cells of neural lineage.⁴⁵ LP-MSCs, however, do express nestin *in vitro*, suggesting that the use of nestin immunoreactivity as a stem cell marker applies specifically to LP-derived neural stem cells.^{37–39}

Adult human OM-derived cells, originating from non-dissociated biopsy tissue and cultured in a monolayer in serum-containing media, are capable of expressing mRNA transcripts or

Box 1 *In vitro* culture paradigms of adult human olfactory mucosa (OM)-derived cells

Human OM is harvested from the nasal septum or turbinates typically by biopsy, or also by exfoliation. Cells can be subsequently propagated from the olfactory neuroepithelium (ON),⁵⁷ its underlying lamina propria (LP)⁶⁰ or from tissue containing both regions.³⁰ Protocols have been established to enhance the neural stem cell properties of OM cells through neurosphere^{57,61} and monolayer culture.⁶²

(A) Organotypic-monolayer cultures: Non-dissociated adult human ON biopsy tissues, containing a portion of underlying LP, can be utilized for propagating OM cells in monolayer cultures in serum-containing media on uncoated or fibronectin-coated plates^{30,60,62,63} or on a Matrigel substrate.^{22,64} In cultures on fibronectin-coated plates, epithelial cells first grow out from biopsy tissue to form sheets, over which grow round, more rapidly proliferating cells after 24–48 h.^{30,60}

(B) Neurosphere cultures: Multiple groups have reported that cells collected from the human ON itself (with or without some underlying LP) can be cultured in neurospheres from dissociated biopsy tissue. In serum-containing media, when adult human ON tissue (without underlying LP) is first dissociated, cells with the bipolar morphology of neurons, along with presumptive olfactory ensheathing cells, are present.⁶¹ Such cells soon disappear in serum-containing media, and are replaced by a proliferating population, which form spheres and can be cultured for extended periods.^{61,65–67} Alternatively, adult human OM-derived cells can be dissociated and propagated on poly-L-lysine-coated plates in defined medium with EGF and FGF2 to form 'primary neurospheres', before being plated onto uncoated or fibronectin-coated plates and maintained in serum-containing medium in monolayer culture.^{16,57}

Additional approaches that have been employed in rodent studies include culture of adult rat ON-derived cells on a feeder layer of newborn rat glial cells,⁶⁸ and co-culture of ON-derived cells with a 3T3 cell feeder layer at the air–liquid culture interface.⁶⁹

proteins characteristic of a number of OM cells types *in vivo* (Figure 2). These include NeuroD1, Gap43, TrkB, NCAM, β -tubulin III, OMP, OcNc1 and OR3A1.^{25,30,61,63,66,74} Markers of sustentacular and glial cells, such as Sus4, S100 and GFAP, and mesenchymal stem cells (Stro-1) have also been reported to be expressed in human OM culture cells (Figure 2).^{30,36–38,49,66} Some ON-derived cells are also capable of progressing from replication (indicated by BrdU incorporation) to terminal differentiation (OMP expression) in culture.³⁰ At a functional level, some human ON cells cultured under these conditions demonstrate the odorant responsiveness specific to OSNs, with ~5.5% of cells in culture responding to helional, a ligand for the OR3A1 odorant receptor,⁶³ and up to 10% of OMP-positive cells responding to a mixture of other odorants.⁷¹ Receptors for the key neurotransmitters serotonin (5-HT2 receptors), dopamine (D2 receptor) and glutamate (NMDA receptors), which have been identified in cultured ON cells, also display functional activity.⁶³ Benitez-King and colleagues collected nasal exfoliates^{24,25} and reported that the cells harvested this way and propagated in monolayer culture expressed with varying frequencies a number of potential cell type-specific markers (Figure 2) and expressed action potentials.²⁵

Neurosphere-forming cells cultivated from either the ON alone, or from both the ON and LP, express markers such as NCAM, β -tubulin III, TrkA/B, nestin and Map2 (Figure 2).^{16,57,61,65–67} Overall, the expression of ON neuronal lineage markers, and of other putative neuronal markers, suggests that many OM-derived culture cells may be of a neural lineage. To date, although multiple

OM-derived cell culture paradigms have been used in various studies, the relative expression of cell type-specific markers by cells cultured in these paradigms has not been directly compared. When indirectly compared, some similarities between paradigms exist in the expression of multiple markers, such as NCAM and nestin (Figure 2), suggesting a degree of consistency among paradigms employing serum-containing media. However, differences also exist, such as in β -tubulin III, which is expressed in 35% of ON-derived cells in the study of Matigian *et al.*,¹⁶ in the majority of cells in multiple other studies^{57,61,67} and in ~100% of ON-derived cells in the study of Benitez-King *et al.*²⁵ Overall, further work to delineate the degree of consistency between culture paradigms, including characterization of cellular heterogeneity and marker expression, is needed.

The adult human olfactory LP alone can also be used to culture progenitors of ectomesenchymal origin, which are particularly suitable for the *in vitro* propagation of presumptive OECs.^{38,75} When cultured in serum-free media supplemented with NT3, almost all adult human LP-derived proliferating cells express OEC markers S100 and GFAP, ~80% express p75NGFR, whereas none express HNK1 (a marker for Schwann cells).^{59,75} These cells hold promise for autologous transplantation in the treatment of spinal cord injury.^{50,59,76}

In vitro culture of OM-derived cells from rodents and fetal humans The OM paradigm has been employed using *in vitro* cells from rodents, shedding light on the stem cell potential of OM-derived cells. For example, proliferation of neuronal precursors and successful genesis of mature OMP-positive OSNs from adult rat ON-derived cells in culture has been reported.⁶⁸ When adult rodent ON-derived cells are co-cultured with a 3T3 cell feeder layer at the air–liquid culture interface, cells with the molecular phenotypes of GBCs, HBCs, sustentacular cells and neurons can be generated, which can successfully reintegrate into the ON after transplantation.⁶⁹ Monolayer culture of rat p75NGFR-expressing OM-derived cells, which were purified by fluorescence-activated cell sorting, has been used for characterization of putative OECs *in vitro*.⁷⁷ Cells from the mouse olfactory LP, when cultured in a monolayer, demonstrate the same OEC phenotype and cell type-specific marker expression as human LP-derived cells.³⁶ When cultured in spheres, mouse olfactory LP-derived cells have the capacity to integrate into lesioned hippocampus *in vivo*, differentiate into neurons and restore learning and memory.⁷⁸

Numerous studies have used OM-derived cells from pre-natal/neonatal rodents^{79–88} and fetal humans,^{89,90} in part because such cells can be propagated more readily. This is observed under controlled neurosphere assay conditions, in which ON-derived neurospheres are generated with progressively lower efficiencies from E13.5 mice, P5 mice and adult mice.³⁵ Importantly, neurospheres derived from E13.5 and adult animals contain cells with different morphologies, which express different cell type-specific markers and proliferate at different rates.⁹¹ As a result, the extent to which findings in OM-derived cells from neonatal and early postnatal animals can be extrapolated to adults may be limited.

The effects of culture environment on neuronal identity of OM-derived cells

The properties of OM-derived cells, such as their proliferation, morphology and expression of cell type-specific markers, can be heavily influenced by the *in vitro* environment, particularly culture medium^{57,60,61,65,66} (Table 1). In culture, BDNF (brain-derived neurotrophic factor), NT3 and NT5 promote ON-derived cell survival,⁹² FGF2, FGF8, EGF and TGF- α (transforming growth factor-alpha) have been used to stimulate progenitor proliferation,^{82,93,94} and serum deprivation, Db-cAMP, IGF-I (insulin-like growth factor-I), retinoic acid, forskolin and sonic hedgehog have been used to drive human ON culture cells toward a

OM CELL TYPE	IN VIVO MARKER	EXPRESSED IN VITRO?	Monolayer cultures		Neurosphere cultures	
			Human	Rodent	Human	Rodent
Basal multipotent progenitor	Sox2, Pax6, CK5,14	Sox2 CK5,14				some ^{79,80}
Neural progenitor	Ascl1, Neurog1	Ascl1 Neurog1		some ⁸²		some ⁸⁰ some ⁷⁹
INP	NeuroD1	NeuroD1 TrkA	some ³⁰		some ⁶¹	
Immature OSN	GAP-43 β-tubulin III, NCAM, MAP1B, PGP9.5	GAP43	some ³⁰			
		β-tubulin III [#]	up to 100% ^{15,25,30,71}	some ^{69,82}	35 to 100% ^{16,57,61,65,66,67}	some ^{69,79,80}
		NCAM [#]	some ^{71,74}		~20% ^{65,66}	some ^{79,80}
		TrkB	some ⁶³		some ⁶¹	
		G _{olf}	some ⁷¹			
Mature OSN	OMP β-tubulin III, NCAM, MAP1B, PGP9.5	OMP	~2% ^{15,25,30,71}	some ^{85,88}		
		β-tubulin III [#]	up to 100% ^{15,25,30,71}	some ^{69,82}	35 to 100% ^{16,57,61,65,66,67}	some ^{69,79,80}
		NCAM [#]	some ^{71,74}	some ⁸⁸	~20% ^{65,66}	some ^{79,80}
		NTRs-5HT2C,D2R,NR1	some ⁶³			
		ACIII, OcNc1	some ⁶³			
OEC	GFAP, S100β, p75NGFR	G _{olf}	some ⁷¹	some ⁸⁸		
		GFAP	few ^{25,30} , LP~100% ⁵⁹	LP~50% ³⁶	Few ^{57,61,66}	
		S100β	some ⁷¹ , LP~100% ⁵⁹	LP~80% ³⁶		some ⁸⁰
		p75NGFR	some ³⁰ , LP~80% ⁵⁹	LP~80% ³⁶	n.d. ⁶¹	
LP-MSC	Stro-1	Stro-1	some ³⁸		35% ³⁸	some ³⁷
		MSC-CDs ^Δ	most ^{38,39}		most ^{38,39}	
Sustentacular cell	CK18, Sus4, Sus5	CK18		some ^{69,82}		some ^{69,79}
		Sus4				some ⁸⁰
		Sus5		some ⁸²		
Other possible progenitor, neuronal or sustentacular cell markers ^Δ		NF-M/H	some ⁷⁴	some ⁸⁸	some ⁵⁷	
		Nestin	~80% ²⁵	LP some ³⁶	~80% ^{57,65,66,67}	some ^{37,38}
		NeuN	~20% ²⁵			
		MAP2	n.d. ⁷⁴		some ^{61,65,66}	
		Vimentin	~100% ^{25,74}	some ^{82,87} LP some ³⁶		

Figure 2. Expression of putative molecular markers of stem cells, neural progenitors, neurons and glia by various olfactory mucosa (OM) cell types, both in adult OM *in vivo* and in cells generated from adult OM tissues *in vitro*. Studies of OM explant tissues or dissociated cells immediately *ex vivo* are not included. Percentage values are approximate, and refer to the proportion of cells in culture which express each marker. Values are only shown for markers displaying consistency between studies, and only for cells grown in serum-containing media. *, studies using neonatally-derived rodent or fetally-derived human ON culture cells; ^Δ, markers whose significance in OM culture cells is uncertain due to a discordance between human and rodent (for example, p75NGFR), low/uncertain levels of expression in adult ON *in vivo* (NF-M/H, Nestin, NeuN, MAP2) or a possible lack of cell type specificity (p75NGFR, Vimentin); #, markers whose expression increase when cells are cultured in base (unsupplemented defined) media rather than serum-containing media; ^Δ, mesenchymal stem cell cluster of differentiation (MSC-CD) markers, used to confirm cell type, vary among studies. CK, cytokeratin; INP, intermediate neural progenitor; LP, lamina propria (indicates cultures derived solely from LP, without ON); LP-MSC, lamina propria mesenchymal stem cell; ND, not detected; NTR, neurotransmitter receptor; OEC, olfactory ensheathing cell; ON, olfactory neuroepithelium; OSN, olfactory sensory neuron.

neuronal phenotype.^{25,95,96} Proliferation of human LP-derived cells with an OEC phenotype is promoted by NT3, BDNF or NGF.⁷⁵ Human LP-derived cells can be driven toward a neuronal phenotype with a cocktail of retinoic acid, forskolin and sonic hedgehog,⁹⁷ or dexamethasone, insulin, 3-isobutyl-1-methyl-xanthine, indomethacin and ethanol,⁹⁸ and toward an osteoblast phenotype with a cocktail of dexamethasone, L-ascorbic acid-2-phosphate, and β-glycerophosphate⁹⁸ or NaH₂PO₄.³⁹ Other additional factors, many of which are relevant to the control of neurogenesis and differentiation *in vivo*, have also been shown to modulate OM-derived cell proliferation, morphology and expression of cell type-specific markers *in vitro* (Table 1).

THE USE OF OM-DERIVED CELLS IN PSYCHIATRY AND NEUROSCIENCE RESEARCH

Historically, the tools to study neural function and mechanisms of drug action in humans at a cellular level have proved elusive. OM-derived cells have emerged as a promising platform for such

investigations in a range of neuropsychiatric, neurodegenerative and developmental disorders.

The ability to investigate functional cellular characteristics represents a distinctive opportunity afforded by the OM-derived cell paradigm. The processes of cell proliferation, adhesion, motility, metabolism and apoptosis have been investigated by Mackay-Sim and colleagues, who showed that ON-derived cells from schizophrenia patients (but not bipolar disorder patients), when cultured in a monolayer from non-dissociated biopsy tissue, proliferate more rapidly than ON-derived cells from healthy controls.^{14,15,17} Such cells were also reported to adhere less effectively to the culture plate than those from controls, both during establishment of the initial explant¹⁵ and during subsequent monolayer culture.¹⁸ This abnormal adhesion was accompanied by increased cell motility, decreased vinculin-positive adhesion complexes and faster disassembly of focal adhesions in schizophrenia ON-derived cells than controls.¹⁸ Metabolic activity of ON-derived cells has been investigated in schizophrenia and Parkinson's disease, and was reduced in

Table 1. *In vivo* and *in vitro* factors influencing the proliferation, differentiation and survival of cells within, or derived from, the olfactory mucosa (OM)

Factor	Cell type impacted in vivo	Effect observed in vivo	Effect on OM-, ON- or LP-derived cells in vitro
TGF- α	Stem cell	Stimulates proliferation and progression of rodent HBCs ⁹⁹	Stimulates ON-derived cell (HBC) proliferation ⁹³
EGF	Stem cell	Stimulates proliferation of rodent HBCs ⁹⁹	Stimulates ON-derived cell (HBC) proliferation ⁹³
Δ Np63 (p63)	Stem cell	Required for rodent HBC genesis and differentiation ¹⁰⁰	—
Sox2	Stem cell	Increases Ascl1 expression and differentiation of rodent GBC/HBCs into neural progenitors ⁹⁴	—
Meis1	Stem cell	Suppresses Ascl1 expression and stem cell differentiation ⁹⁴	—
Foxg1	Stem cell	Promotes differentiation of rodent Sox2-positive stem cells into neural progenitors ¹⁰¹	—
Retinoic acid	Stem cell	Stimulates basal cell proliferation and/or differentiation after lesion ¹⁰²	Promotes ON-derived cell (HBC) differentiation ⁹⁵ and LP-derived neuronal differentiation ³⁷
Uncx	Early neural progenitor	Promotes proliferation of neural progenitors and survival of OSNs ¹⁰³	—
FGF2	Early neural progenitor	Enhances neural progenitor proliferation ¹⁰⁴	Stimulates OM-derived cell proliferation ^{37,83,90,105}
FGF8	Early neural progenitor	Promotes neurogenesis and progenitor proliferation ¹⁰⁶ by enhancing Sox2 and repressing Meis1 expression ⁹⁴	—
Ascl1	Early neural progenitor	Required for maturation of early neural progenitors into INPs ^{107,108}	—
Six1	Early neural progenitor	Required for maturation of early neural progenitors into INPs ¹⁰⁹	—
Ngn1	Early neural progenitor	Required for maturation of neural progenitors into INPs ¹⁰⁸	—
BMP2,4,7	Early neural progenitor, immature OSN	At lower concentrations, promotes survival of newly formed OSNs (BMP4 only), ¹¹⁰ at higher concentrations inhibit neurogenesis by targeting Ascl1 for proteolysis ¹¹¹	—
FST	Early neural progenitor	Promotes progenitor proliferation by antagonizing GDF11 and ACT β ^{112,113}	—
ACT β B (activin)	Early neural progenitor	Inhibits progenitor proliferation by promoting proteolysis ¹¹³	—
Hes1	Early neural progenitor	Suppresses Ascl1-mediated neural progenitor maturation ^{114,115}	—
Hes5 (with Hes1)	Early neural progenitor	Suppresses Ngn1-mediated neural progenitor maturation ^{114,115}	—
MMP2, MTI-MMP	Early neural progenitor, INP	Downregulated following bulbectomy in rodent GBCs ¹¹⁶	—
TIMP	Early neural progenitor, INP	Upregulated following bulbectomy in rodent GBCs ¹¹⁶	—
GDF11	INP	Inhibits proliferation of INPs by inducing reversible cell cycle arrest ^{112,113}	—
Runx1	INP	Stimulates INP proliferation ¹¹⁷	Stimulates ON-derived cell proliferation ¹¹⁷
NPY	INP	Promotes, and is required for, INP proliferation and maturation ^{118–121}	Increases OM-derived cell proliferation via the Y1 receptor and PKC-dependent ERK1/2 phosphorylation ^{119,121}
Peptide YY	INP	Regulates INP differentiation ^{118,119}	Increases OM-derived cell proliferation ^{118,119}
Atf5	Immature OSN	Promotes survival and maturation of immature OSNs ¹²²	—
Emx12	Immature OSN	Promotes survival and maturation of immature OSNs ¹²³	—
Lhx2	Immature OSN	Promotes survival and maturation of immature OSNs ^{124,125}	—
ZFP423/OAZ	Immature OSN	Inhibits maturation of immature OSNs ¹²⁶	—
LIF1	Immature OSN	Promotes survival and inhibits maturation of immature OSNs ^{127,128}	Increases OM- and ON-derived cell proliferation, ^{93,129} decreases apoptosis, inhibits differentiation ^{127,128}
BDNF	Multiple cell types	May provide trophic support to maturing ON cells ^{130–132}	Promotes LP-derived glial cell proliferation ⁷⁵ and neuronal differentiation ³⁷
NT3,4	Multiple cell types	May provide trophic support to maturing ON cells ¹³¹	(NT3) promotes LP-derived glial cell proliferation ⁷⁵
NGF	Multiple cell types	Expressed in neuronal ON layers, may provide trophic support to maturing ON cells ¹³¹	Promotes LP-derived glial cell proliferation ⁷⁵
PACAP	—	—	Promotes proliferation of OM-derived cells ¹³³
IGF-I	—	—	Decreases OM-derived cell proliferation and promotes cell differentiation ⁹⁶
Nitric oxide	—	—	Inhibits ON-derived cell proliferation and promotes differentiation ¹³⁴
Serum deprivation	—	—	Decreases ON-derived cell proliferation and increases neuronal differentiation ^{66,82}

Table 1. (Continued)

Factor	Cell type impacted in vivo	Effect observed in vivo	Effect on OM-, ON- or LP-derived cells in vitro
Db-cAMP	—	—	Promotes ON-derived cell differentiation ^{25,61}
Forskolin	—	—	Promotes ON-derived cell differentiation ⁹⁵
Sonic hedgehog	—	—	Promotes ON-derived cell (HBC) differentiation ⁹⁵

Abbreviations: BDNF, brain-derived neurotrophic factor; GBC, globose basal cell; HBC, horizontal basal cell; IGF-I, insulin-like growth factor-I; INP, intermediate neuronal precursor; LP, lamina propria; NGF, nerve growth factor; ON, olfactory neuroepithelium; OSN, olfactory sensory neuron; TGF- α , transforming growth factor- α .

Parkinson's disease.¹⁶ Increased cell death has been observed in ON-derived cells from bipolar disorder patients (but not schizophrenia patients), relative to healthy controls,¹⁴ although a subsequent study reported increased caspase-3/7 activity in ON-derived cells from schizophrenia patients, suggesting increased apoptotic activity.¹⁶

Cellular processes governing microtubule formation and cytoskeletal structure have also been investigated in OM-derived cells from individuals with psychiatric illness. Dynamic structural processes have been monitored across time in ON-derived cells, and increased microtubule stability in ON-derived cells from individuals with schizophrenia than in ON-derived cells from controls has been reported.¹³⁵ In addition, in a paradigm employing exfoliation and monolayer culture, ON-derived cells from schizophrenia patients were described by Benitez-King and colleagues to display abnormal microtubule organization, whereas ON-derived cells from bipolar disorder patients displayed decreased microtubule length and cytoplasmic β -tubulin III protein expression.^{24,25} Decreased L-type voltage-activated calcium currents in the ON-derived cells from individuals with schizophrenia were also reported in this study.²⁶ Overall, these studies suggest the potential utility of OM-derived cells for the investigation of cellular function in neuropsychiatric disorders. The reported findings in OM-derived cells implicate processes and pathways, which have been independently implicated in schizophrenia and bipolar disorder by postmortem studies, such as in neurogenesis,^{136,137} neuronal migration^{137,138} and apoptosis.¹³⁹ However, they also hold the promise of revealing aspects of neural cell function in psychiatric illness that are difficult to investigate using traditional approaches.

The OM-derived cell culture approach also provides a powerful platform to explore mechanisms of drug action, and to target pharmacologically the molecular pathways of interest in psychiatric disorders. Studies have been performed investigating the effects of pharmacological agents on intracellular signaling, molecular expression and cell function in ON-derived cells derived from schizophrenia, bipolar disorder and major depressive disorder patients. Feron *et al.*¹⁴⁰ demonstrated that dopamine increases apoptosis in control ON-derived cells, but decreases apoptosis in schizophrenia cells. Cellular effects of lithium, a first-line bipolar disorder treatment, have also been explored in ON-derived cells, revealing amelioration by lithium of ionic stress-induced apoptosis and TRPM2 overexpression.¹⁴¹ In major depressive disorder, there is evidence that ON-derived cells display decreased nuclear translocation of the glucocorticoid receptor, GR, in response to glucocorticoids relative to controls.¹⁴² This diminished receptor translocation was accompanied by greater cytoplasmic association of GR with the immunophilin FKBP51.¹⁴² These studies suggest the potential utility of ON-derived cells for understanding current treatments and developing novel pharmacological agents in psychiatry.

The experimental potential of OM-derived cells from psychiatric patients has also been highlighted by studies of subcellular molecular abnormalities. As in work with *ex vivo* ON tissue, many

molecular and cellular studies using ON-derived cells have demonstrated a concordance with postmortem brain studies. ON-derived cells from Alzheimer's patients exhibited increased amyloid precursor protein levels,¹⁴³ along with other protein alterations,²³ consistent with changes in multiple brain regions in the illness.⁵² Dysregulation of gene expression in multiple cellular pathways, including cell cycle regulation,^{14,17} oxidative stress response,^{16,144} focal adhesion¹⁶ and axon guidance¹⁶ have been reported in ON-derived cells from schizophrenia patients, some of which may be mediated by altered histone H3 lysine 4 trimethylation.¹⁴⁴ These findings are consistent with postmortem brain studies in schizophrenia showing aberrant histone H3 lysine 4 trimethylation within the GAD1 gene¹⁴⁵ and dysregulated expression of immune genes.^{146,147} Protein changes in oxidative stress and cell adhesion pathways have also been recently reported in a preliminary human iPSC study.¹⁴⁸ As described earlier, increased expression of miR382 has been reported in ON-derived cells from individuals with schizophrenia,²¹ mirroring findings in *ex vivo* microdissected ON tissue²¹ and postmortem prefrontal cortex.⁵³ In ON-derived cells from bipolar disorder patients, gene expression changes within the phosphatidylinositol signaling pathway¹⁴ have been reported. In Alzheimer's and Parkinson's diseases, OM-derived cells have also demonstrated altered gene and protein expression profiles, implicating mitochondrial function, oxidative stress and xenobiotic metabolism in these illnesses.^{16,22} Finally, OM-derived cells have also yielded insights into molecular pathologies in developmental disorders. In an early study interpreted as suggesting blood-brain concordance of FMRP deficits in fragile X syndrome, decreased FMRP protein was observed in ON-derived cells from affected individuals.¹⁴⁹ LP-MSCs from individuals with familial dysautonomia, which is caused by mutations within the IKBKAP gene, display lower IKBKAP mRNA expression, decreased IKAP/hELP1 protein, altered IKAP/hELP1 subcellular localization and dysregulation of genes involved in cell migration and cytoskeletal reorganization.⁹⁷ Overall, these studies indicate the potential for OM-derived cells to shed light on molecular abnormalities associated with brain disorders, and the concordance of many OM-derived cell studies with postmortem findings highlights the potential for OM-derived cells to reflect pathophysiological mechanisms in the brain.

CONCLUSIONS

Advantages and challenges for olfactory paradigms in the study of neuropsychiatric illnesses

Olfactory mucosal biopsy studies afford a unique opportunity in neuropsychiatric research, offering *ex vivo* and *in vitro* neural cells from living individuals, which harbor biological characteristics that may be more relevant to neuropsychiatric disease than blood cells¹⁵⁰ or skin fibroblasts. Figure 3 provides a summary of the previous and potential uses of OM-derived cells in psychiatric and neuroscience research, as described throughout this review.

The OM model has a number of strengths. First, *ex vivo* OM tissues were previously exposed to the *in vivo* neurohormonal

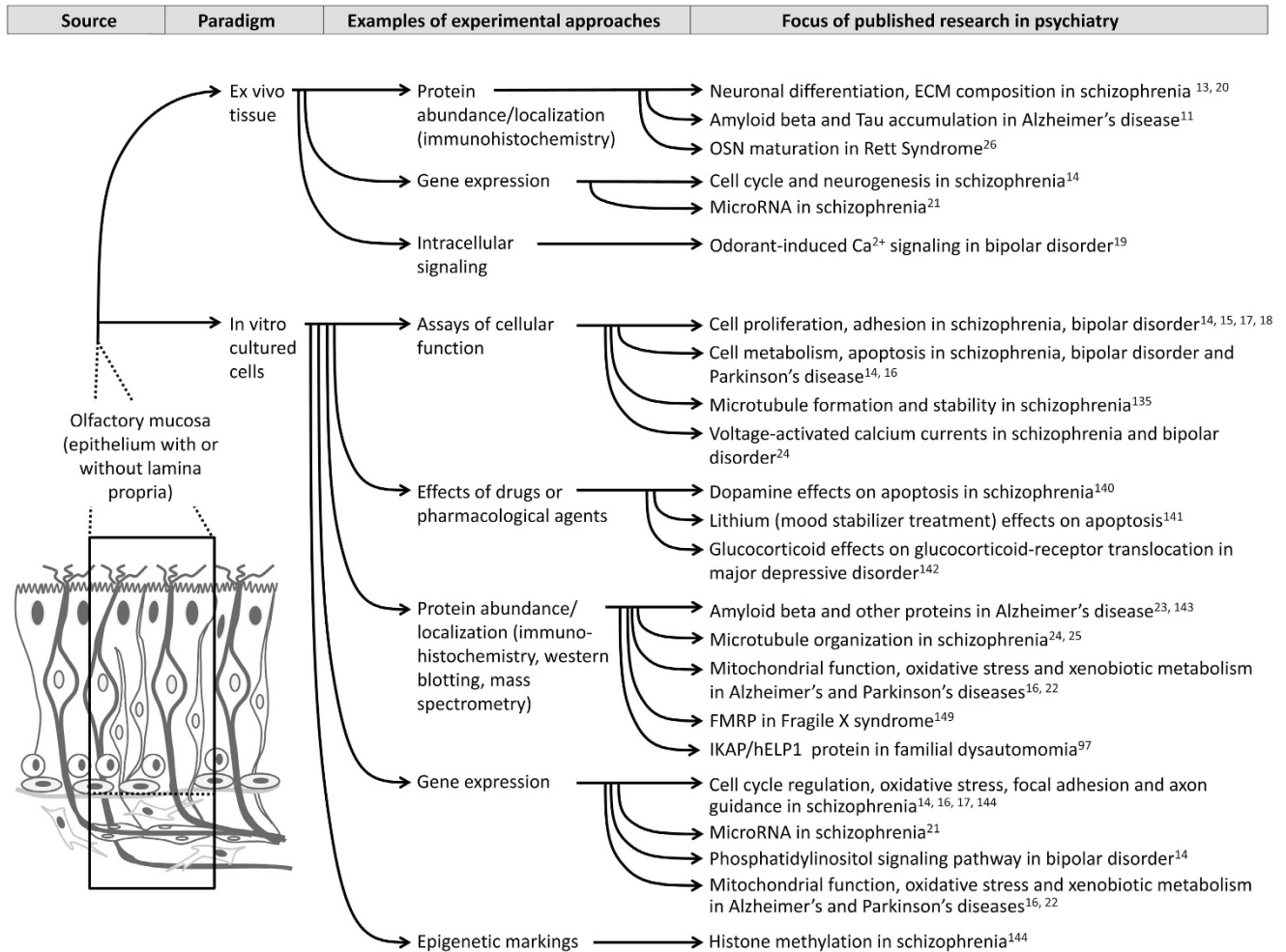


Figure 3. Examples of the use of *ex vivo* olfactory tissues and *in vitro* OM-derived cells in psychiatric research.

milieu and contain neurobiological signatures of the *in vivo* condition. As such, they can serve as a point of reference for *in vivo* and *in vitro* OM findings, bridging the gap between these two approaches. Second, OM biopsies, though limited by the quantity of tissue available, can be safely obtained from the same subjects multiple times (monthly, up to three consecutive times in our human subjects protocols) and can be fitted with longitudinal clinical study designs. In this approach, OM biopsies are obtained in specific phases of the illness, while patients' clinical conditions are carefully characterized. Interpretation of neurobiological parameters in the context of clinical changes is a rare opportunity in neuropsychiatric research. Examples of such use include but are not limited to the collection and examination of OM biopsies from (1) individuals at risk for schizophrenia and unaffected relatives before and after the onset of illness, (2) mood disorder patients before, during and after disease episode (such as in depressive disorders or bipolar disorder), (3) any neurodevelopmental or psychiatric disease before, during or after treatment. Finally, one promising aspect of using *ex vivo* OM tissues in psychiatric illness involves their relation with the broader olfactory neurocircuitry: olfactory mucosa → olfactory bulb → olfactory cortex. Disease-associated alterations within the ON may not occur in isolation, and may reflect brain abnormalities within the broader olfactory neurocircuitry. Indeed, numerous structural and functional decrements associated with neuropsychiatric diseases are reported in many of these brain regions. Olfactory circuitry can now be interrogated by psychophysical testing, electroolfactogram and

evoked potentials. Combining these measures with molecular and cellular measures derived from OM biopsy tissues can help us to link molecules to cellular changes and then to circuit activity.

An important consideration in using *ex vivo* tissues is that the human ON is patchy and thus some biopsy tissues may not contain OSNs. Approximately 70 to 80% of ON biopsy tissues obtained from nasal septum or middle turbinate yield olfactory receptor neurons when dissociated immediately from the biopsy (unpublished observations). This is an important consideration, however, particularly for histologic examination of OE tissues. As the neuroepithelial patches are intermixed with respiratory epithelium, quantitative assessment of protein immunoreactivity or mRNA needs to be conducted by delimiting neuroepithelial versus respiratory patches in histologic sections. Under *in vitro* conditions, however, such limitations do not appear to figure greatly into the overall cellular composition of resultant culture cells. Nonetheless, quantitative assessment of cell type-specific proteins, whether neuronal, glial or epithelial, should be a guide for including cell lines for between-group comparison studies.

As noted in the preceding sections, the *in vitro* ON paradigm is particularly informative in studies of active cellular processes and pathways, where pharmacologic manipulations are to be tested or where larger numbers of cells from an individual are required. Importantly, as these cells are of neuronal and glial lineages they better represent the molecular dysregulations of neuropsychiatric disease than those of other cell types used in *in vitro* studies, such as blood cells. Indeed gene expression profiles of cells derived

Table 2. General comparison of some characteristics of olfactory mucosa (OM)-derived, induced pluripotent (iPS) and induced neural (iN) stem cells relevant to the use of these cells in the study of psychiatric, neurodevelopmental and neurodegenerative disorders

Characteristic	Adult human OM-derived cells	Adult human iPS cells	Adult human iN cells
Ease of collection	Moderate ^a	High	High
Current cost to prepare cells for assay	Low–moderate	High	Moderate
Extent of <i>in vitro</i> modification	Little modification: only the influence of <i>in vitro</i> conditions	Substantial modification: reprogramming for pluripotency plus the influence of <i>in vitro</i> conditions	Substantial modification: reprogramming for somatic conversion plus the influence of <i>in vitro</i> conditions
Availability of <i>ex vivo</i> neural reference tissue	Yes	No	No
Proliferative capacity of cells used for assays	Moderate–high	Moderate	None
Capacity to be driven to neuronal differentiation	Varying between protocols	Robust, high efficiency	Robust, moderate efficiency
Cellular phenotype commonly used in assays	Proliferative putative neural progenitor cell	Differentiated neuron	Differentiated neuron
Action potential generation	To be further demonstrated ²⁵	Yes	Yes

Detailed discussion of iPS and iN cell paradigms is beyond the scope of this review, but these paradigms have been reviewed extensively elsewhere.^{1,2} See text for details and references for OM-derived cell studies. ^aCollection of OM tissues is performed by a qualified otorhinolaryngologist using local anesthetic.

from OM biopsy more closely resemble brain tissue, stem cells and neurons derived from stem cells than blood cells (see Horiuchi *et al.*¹⁵⁰ for a comparison of gene expression profiles of brain tissue, OM cells, stem cells and iPSCs). While iPS and induced neuronal cell paradigms offer unparalleled opportunities to generate differentiated neurons *in vitro* from living individuals, there has been considerably less study of the capacity of OM cells to generate differentiated neurons. Nevertheless, OM cells have distinct advantages (Table 2). The timeline from OM biopsy to availability of proliferated precursor cells is ~4 weeks, whereas iPSC reprogramming protocol timelines are considerably longer, varying on the basis of source cells and methods. In addition, OM cells are readily proliferative and can be rapidly expanded over multiple passages. Thus OM studies have lower costs and require less time and labor (Table 2).

Most importantly, OM tissues are obtained as neural tissues (Table 2) and thus they do not require genomic reprogramming as with iPS or induced neuronal cells. A challenge in the iPS and induced neuronal paradigms is to determine to what degree phenotypes of these cells have been modified compared with those of brain cells. In the OM approach, however, neurobiological properties, such as epigenomic profiles, may be less modified than in the cells genetically reprogrammed. Given that the *ex vivo* tissues are available as the source of *in vitro* cells, the extent of modification could at least be evaluated in OM cells.

There are a number of challenges for researchers working with *in vitro* OM cells. Differences in protocols between research groups must be understood and controlled for, in particular during studies with OM-derived cells since such cells may be susceptible to variation in the means of cell harvest and the culture environment. To what degree OM-derived cells assume characteristics of differentiated neurons or glia is not entirely clear at present. As discussed earlier, for these olfactory paradigms to provide consistent insight into the pathophysiology and treatment of neuropsychiatric illnesses, additional characterization of OM-derived cells in different protocols is required to determine the characteristics of their molecular phenotypes and the similarities and differences between protocols. Ultimately, the key question surrounding the use of OM tissues and OM-derived cells in the study of psychiatric illness will be in what way findings from these studies can shed light on abnormalities in other important brain regions and neural circuits in psychiatric illness. This will involve the examination of olfactory-derived tissues and

function at multiple levels of the olfactory circuit, as well as direct parallels between OM findings and brain pathology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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