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Application of olfactory tissue and its neural progenitors to schizophrenia and psychiatric research

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Abstract

Purpose of review—The goal of this review article is to introduce olfactory epithelium (OE)-derived cell/tissue models as a promising surrogate system to study the molecular mechanisms implicated in schizophrenia (SZ) and other neuropsychiatric disorders. Here we particularly focus the utility of their neural progenitors.

Recent findings—Recent investigations of the pathophysiology of SZ using OE-derived tissue/cell models have provided insights about SZ-associated alterations in neurodevelopment, stress response, and gene/protein expression regulatory pathways.

Summary—The OE retains the capacity for lifelong neurogenesis and regeneration, because of the presence of neural stem cells and progenitors. Thus, both mature neurons and neural progenitors can be obtained from the OE without the need for genetic reprogramming and related confounds. Furthermore, the OE is highly scalable resource in translational settings. Here we also demonstrate recent findings from research using OE-derived tissue/cell models in SZ and other brain disorders. In summary, we propose that the OE as a promising resource to study neural molecular and cellular signatures relevant to the pathology of SZ and other mental disorders.

Keywords

schizophrenia; olfactory epithelium; neural progenitors; cell/tissue models; translational psychiatry

Introduction

Investigating the cellular and molecular mechanisms of neuropsychiatric disorders represents a significant challenge, because of the inability to directly access the central nervous system for study. Although the exact pathophysiology of schizophrenia (SZ) remains elusive, the general consensus is that it involves a complex interplay between multiple genetic and environmental risk factors [1]. Several lines of evidence support that SZ substantially shares common biological deficits with other psychiatric conditions [2]. For example, psychosis or cognitive deficits that are prominent in patients with SZ also appear in mood disorders and neurodegenerative diseases [3].

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Conflicts of interest: None.

In order to unravel the biological underpinnings of SZ, as well as other major neuropsychiatric disorders with common deficits, several types of human biospecimens are currently being used for research, including postmortem brains, cerebrospinal fluid, blood, fibroblasts, and genetically engineered cells, such as induced pluripotent stem (iPS) cells and induced neuronal (iN) cells. In addition to these models, the olfactory epithelium (OE), containing mature olfactory neurons and neural progenitors, has been used in SZ research. An advantage of using the OE is that there is no need for genetic reprogramming to obtain neurons and neural progenitors, which promises high scalability and avoids any confounds related to reprogramming in translational settings [4, 5].

Olfactory dysfunctions are commonly observed in patients with SZ, as well as in their first-degree relatives and subjects with at-risk mental state. These deficits are present early in the course of the illness [6-11]. Therefore, olfactory dysfunctions may represent one of the intrinsic traits associated with SZ, and have been proposed to be an endophenotype or a pre-morbid marker for SZ [12, 13]. Since several brain areas (e.g., the ventromedial temporal cortex and prefrontal cortex) mediate olfactory processing, one may hypothesize that the functional deficits may be attributed at least in part to such central abnormalities [14]. Meanwhile, as olfactory cells from the OE of SZ patients also present particular molecular and cellular deficits [15-18], the peripheral olfactory system may also contribute to these dysfunctions. Furthermore, the molecular and cellular deficits seen in olfactory neurons and progenitors in the OE may represent general (or default) neural abnormalities and vulnerabilities [19]. Thus, we propose the OE-derived cell/tissue models as a promising surrogate system to study the molecular mechanisms implicated in SZ.

Neural progenitors in the OE

The OE and underlying lamina propria consist of olfactory mucosa, typically located dorsally and posteriorly in the nasal cavity in mammals [20]. The OE is a pseudostratified epithelium that contains several types of cells, including olfactory receptor neurons (ORNs), basal cells, and sustentacular (supporting) cells [21] (Fig. 1). ORNs are sensory neurons that project their axons onto the olfactory bulb via the olfactory nerve. Mature and immature (transitional) ORNs are located in the intermediate layer of the OE, and these cells are continuously replaced by neurogenesis throughout adult life [21]. Interestingly, this process is regulated by the same growth factors that control neurogenesis in the central nervous system, such as the epidermal growth factor (EGF) and fibroblast growth factor (FGF2) [22].

The basal layer, which is adjacent to the basal membrane, contains basal cells [21, 23, 24]. The OE retains the capacity for lifelong neurogenesis and regeneration, because of the presence of these neurocompetent stem and progenitor cells [23-25]. In the mouse, it is well known that there are two types of basal cells, horizontal basal cells (HBCs) and globose basal cells (GBCs) [19]. Although it has been suggested that both HBCs and GBCs are capable of functioning as neurocompetent, broadly multipotent progenitors, HBCs are considered to be the “reserve” stem cells of the adult OE. In contrast, GBCs are a more active progenitor population responsible for day-to-day replacement of neurons, as well as reconstituting both neuronal and non-neuronal populations of the epithelium following injury [25]. In the human, however, the composition of the basal cell population has been

subject to considerable debate. The topological, morphological, and molecular expression pattern differences between two cell types in the human OE appear to be less clearly defined, compared to those in the mouse OE [23, 24].

OE-derived tissue/cell models

Thus far, multiple protocols have been developed to model the pathophysiology of brain disorders by using OE- derived tissue/cells [4, 5, 19, 26, 27] (also see Table 1). However, a systematic comparison of observations obtained from different protocols for the OE has not been made. To organize these findings, we categorize OE- derived tissue/cell models into two groups in this review: 1) olfactory neurospheres and their derived cells; and 2) non-neurosphere approach, including other OE-derived cultured cells and tissue. Note, olfactory neurospheres are defined as cellular spheres formed from olfactory stem/progenitor cells in olfactory mucosa/epithelium cultures [28, 29].

1) Olfactory neurospheres and their derived cells

Neural stem/progenitor cells from the forebrain typically grow *in vitro* as spheres, and are used as a surrogate cell type to study neural stem/progenitor cells [30]. Similarly, culture techniques have been established for human OE to generate populations of mitotically active neural progenitors that form neurospheres [28, 29, 31]. The resulting olfactory neurospheres and their derived cells are multipotent and capable of self-renewal, and can be differentiated into neurons and glia [29, 32]. Although there are several protocols to obtain neurospheres from a nasal biopsy, a protocol from Mackay-Sim's group has been most intensively used in SZ research [15, 33-36].

Briefly, olfactory mucosa biopsies including the OE and lamina propria are dissociated and grown for 3 days in Dulbecco's modified Eagles medium (DMEM)/F12 supplemented with 10% fetal bovine serum (FBS). Then, the primary cultures are passaged into flasks coated with poly-L-lysine, and incubated in DMEM/F12 containing EGF (50 ng/ml) and FGF2 (25 ng/ml). Once cell clusters attach to the culture dish, some cells float from the surface to form neurospheres. The free-floating neurospheres are then harvested, dissociated, and expanded in DMEM/F12 with 10% FBS (neurosphere-derived cells) [15, 33-36]. Human olfactory neurosphere cultures express cell markers including CD105 (mesenchymal stem cell marker), CD73 (mesenchymal stem cell marker), organic cation/carnitine transporter 4 (OCT4) (embryonic stem cell marker), Nestin (neural stem cell marker), and β -tubulin III (immature neuron marker) [34]. Importantly, their gene expression profiles are stable between passages 7 through 25, and at more than 100 days *in vitro* [34]. Note, the olfactory lamina propria dissected from the olfactory mucosa also forms spheres and gives rise to bone cells and neurons. Indeed, olfactory lamina propria stem cells share membrane markers with bone marrow stem cells, and are named, olfactory ectomesenchymal stem cells [37]. Other groups have demonstrated that neurosphere-forming cells derived from the OE can be directed toward neuronal lineage restriction by sonic hedgehog, forskolin, and retinoic acid [38], and that human olfactory spheres express oligodendrocyte progenitor cell markers [31].

2) Non-neurosphere approach

Nasal biopsies can be also used to establish dissociated, adherent cultures without sphere formation or explant cultures [17, 39-41]. One protocol for dissociated cultures can purify homogenous, β -tubulin III-positive cell populations through re-plating process to remove possible contamination by unwanted cell types. Such olfactory cells have shown similar gene expression profiles to stem cells and brain tissues, but not blood cells [42].

The whole or dissected OE tissue can also be used to address anatomical and molecular questions related to brain diseases [11, 18, 43], but it is difficult to utilize the OE tissue models for functional assays [4]. However the tissue models can address state changes associated with disease [5]. To selectively enrich the neural population, the neural layer can be isolated from the OE tissue by combining nasal biopsies with laser-capture microdissection (LCM) [43, 44].

Findings from OE-derived tissue/cell models obtained from SZ patients (Table 1)

1) Olfactory neurospheres and their derived cells

Using microarray gene expression profiling, olfactory neurosphere-derived cells from 9 medicated SZ patients showed dysregulation of genes involved in brain development, specifically in focal adhesion, axonal guidance, and receptor-mediated signalling, relative to cells from 14 healthy controls. Importantly, five of the identified pathways had already been implicated in SZ pathophysiology (reelin signalling in neurons, vitamin D receptor and retinoid X receptor activation, ErbB signalling, interleukin-8 signalling, and glutathione metabolism) [34].

A discovery-based proteomics approach using olfactory neurosphere-derived cells from the same 9 medicated SZ patients and 9 of the above 14 controls demonstrated that several proteins were differentially expressed in SZ relative to control cells, particularly those involved in the eukaryotic initiation factor 2/4 (eIF2/4) and mechanistic target of rapamycin (mTOR) pathways. SZ cells also showed decreased expression of 17 ribosomal proteins, which was associated with reduced rate of protein synthesis, as well as dysregulation of cytoskeletal, mitochondrial, and calcium binding proteins compared to control cells [15]. Interestingly, dysregulation of the eIF2 and mTOR pathways was also reported in an iPS cell model derived from SZ patients [45].

Olfactory neurosphere-derived cells from these 9 SZ patients showed increased cell proliferation within 96 hours and cell cycle entry within 30 hours compared to the 9 control cell lines. Also, cell cycle phases were advanced by 2 hours, and the cell cycle period was 2 hours shorter in patients' cells compared to control cells. Interestingly, these cell cycle phenotypes were associated with increased expression of cyclin proteins D1 (CCND1), E (CCNE), and A2 (CCNA2) in SZ cells. Thus, dysregulated cell cycle dynamics may alter the temporal and spatial cascade of brain development, and contribute to an altered neurodevelopmental trajectory in SZ [35].

Finally, altered focal adhesion dynamics were reported in these cells. The focal adhesion kinase (FAK) signalling pathway plays a major role in regulating the formation of focal adhesions and actin stress fibers, and is involved in neuronal migration during brain development [36]. Integrins are also involved in adhesion to the extracellular matrix during cell migration [36], and SZ olfactory neurosphere-derived cells showed increased expression of integrin subunit alpha 8 (*ITGA8*) relative to control cells [34]. SZ cells showed reduced phosphorylation of FAK at tyrosine-397 (Y397-pFAK) compared to control cells, and this observation was associated with diminished cell adhesion, as well as increased motility that was reduced to control levels by using antibodies to block integrins $\alpha 8$ and $\beta 1$. Unexpectedly, reducing Y397-pFAK with an FAK inhibitor also decreased the patient cells' motility to control levels. These paradoxical results were discussed as a future topic in this report [36]. Furthermore, patient cells had fewer and smaller component proteins of focal adhesion protein complexes that disassembled faster than in control cells. Thus, FAK signalling is a potential candidate pathway that may affect neuronal migration in the developing brain in SZ [36].

These findings from SZ olfactory neurosphere-derived cells suggest alterations in neurodevelopmental processes that are involved in the pathophysiology of this disease. However, it is important to consider that a limited number of research groups have used this cell model in a small number of subjects to investigate the pathophysiology of SZ. More studies using this cell model from different groups are warranted to confirm these observations.

2) Non- neurosphere approach

A study using olfactory cells purified through the re-plating technique observed increased expression of miR-382 in SZ relative to control cells. miR-382 was also previously shown to be increased in autopsied brains of SZ patients [46]. Furthermore, these SZ cells showed altered levels of histone methylation in several gene groups involved in cellular protection against oxidative stress, suggesting a potential role of oxidative stress in the pathophysiology of SZ [17]. Cultured cells established via nasal brush swab showed altered microtubular organization, together with decreased function of L-type Ca^{2+} channels in patients with SZ relative to controls [47]. An OE explant (non-dissociated) culture from SZ patients showed increased stability of microtubules and a greater percentage of cells with intact microtubules [40], as well as a higher ratio of mitotic cells to dying and quiescent cells [41, 48]. In addition, this OE explant culture showed differential expression in genes implicated in non-selective vesicular transport, DNA recombination, RNA splicing, and cell proliferation between SZ patients and healthy subjects [48].

Olfactory tissue has provided insights into the neurodevelopmental aspects of the disease. For example, by using neural layers from biopsied OE enriched by LCM, the SMAD pathway was found to be downregulated and potentially associated with cognitive deficits in SZ [16]. A significant decrease in the density of p75-NGFR- positive basal cells in SZ OE nasal biopsies was also observed, suggesting dysregulation in the development of ORNs [11]. A dysregulation of OE neural lineage was also reported in OE tissue obtained at autopsy, as observed in the abnormal densities and ratios of OE neurons at different

developmental stages in SZ samples. It was further suggested that a lack of trophic support from axonal targets in the olfactory bulb, or deficits in intrinsic factors controlling differentiation might contribute to these observations [18]. Finally, abnormalities in chondroitin sulfate proteoglycans expression resulting in disruption of ORNs' axonal outgrowth and guidance were also reported in post-mortem OE tissue from SZ patients [49].

Lessons from other brain conditions (Table 1)

1) Other neurodevelopmental disorders

At the molecular level, it was found that molybdenum cofactor sulfuryase (MOCOS), involved in purine metabolism, was downregulated in nasal olfactory stem cells isolated from nasal biopsies of adults with autism [50]. In Rett syndrome, which is due to a mutation in the methyl-CpG binding protein 2 (*MECP2*) gene, biopsies of nasal epithelium containing ORNs revealed several abnormalities compared to controls, including dysmorphic neurons, as well as an increased ratio of immature to mature neurons [51]. Finally, continuous cell lines of olfactory neuroblasts were used to study the fragile X mental retardation 1 (FMR1) mutation in patients with fragile X syndrome [52].

2) Bipolar disorder (BD)

Some *in vitro* abnormalities were observed in OE-derived neural cells from BD patients, such as alterations in intracellular calcium signaling in biopsy-derived ORNs [53], and a higher ratio of dying to live cells in olfactory neuroepithelial cultures relative to that in control cultures [48]. Moreover, in the latter study, gene expression profiling in olfactory neuroepithelial cultures revealed that 210 genes were differentially expressed between BD patients and controls, with a decreased expression of five genes involved in or related to the phosphatidylinositol signalling [48]. Finally, a platform using nasal biopsies combined with LCM showed the effect of lithium treatment on glycogen synthase kinase-3 beta gene expression [44].

3) Neurodegenerative diseases

At the histological level, several studies reported the presence of typical signs of Alzheimer's disease in the post-mortem olfactory mucosa [54, 55] and cultured olfactory tissue [56, 57], including increased staining for tau, amyloid precursor protein, amyloid- β , apolipoprotein E, neurofilaments, as well as signs of oxidative stress [58- 60]. The use of olfactory neurospheres led to interesting findings about the pathophysiology of Parkinson's disease, including the potential implication of the nuclear factor E2 (NFE2)-related factor 2 (NRF2) signaling pathway [61], as well as dysregulation of pathways implicated in oxidative stress, mitochondrial function, and xenobiotic metabolism [34].

Conclusion

Since the OE retains the capacity for lifelong neurogenesis and regeneration due to the presence of neural stem cells and progenitors, confounds related to genetic reprogramming when establishing iPS cells can be bypassed by using OE-derived neural cells. Furthermore, OE-derived neural cells can be collected longitudinally from human subjects in a relatively

non-invasive manner. Based on these valuable advantages of the OE, OE-derived cell/tissue models have been utilized to uncover neural signatures relevant to the pathology of SZ and other brain disorders in living patients, as a powerful complementary tool to iPS cell-derived models.

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References and recommended reading

1. Owen MJ, Sawa A, Mortensen PB. Schizophrenia Lancet. 2016; 388:86–97. [PubMed: 26777917]
2. Potash JB, Bienvenu OJ. Neuropsychiatric disorders: Shared genetics of bipolar disorder and schizophrenia. Nat Rev Neurol. 2009; 5:299–300. [PubMed: 19498428]
3. Almeida OP, McCaul K, Hankey GJ, et al. Affective Disorders, Psychosis and Dementia in a Community Sample of Older Men with and without Parkinson's Disease. PLoS One. 2016; 11:e0163781. [PubMed: 27689715]
4. Gamo NJ, Sawa A. Human stem cells and surrogate tissues for basic and translational study of mental disorders. Biol Psychiatry. 2014; 75:918–919. [PubMed: 24862565]
5. Lavoie J, Astorga PG, Segal-Gavish H, et al. The olfactory neural epithelium as a tool in neuroscience. Trends Mol Med. in press.
6. Moberg PJ, Kamath V, Marchetto DM, et al. Meta-analysis of olfactory function in schizophrenia, first-degree family members, and youths at-risk for psychosis. Schizophr Bull. 2014; 40:50–59. [PubMed: 23641047]
7. Rupp CI, Fleischhacker WW, Kemmler G, et al. Various bilateral olfactory deficits in male patients with schizophrenia. Schizophr Bull. 2005; 31:155–165. [PubMed: 15888433]
8. Turetsky BI, Hahn CG, Arnold SE, Moberg PJ. Olfactory receptor neuron dysfunction in schizophrenia. Neuropsychopharmacology. 2009; 34:767–774. [PubMed: 18754006]
9. Turetsky BI, Moberg PJ. An odor-specific threshold deficit implicates abnormal intracellular cyclic AMP signaling in schizophrenia. Am J Psychiatry. 2009; 166:226–233. [PubMed: 19074977]
10. Sawa A, Cascella NG. Peripheral olfactory system for clinical and basic psychiatry: a promising entry point to the mystery of brain mechanism and biomarker identification in schizophrenia. Am J Psychiatry. 2009; 166:137–139. [PubMed: 19188289]
11. Minovi A, Dombrowski T, Brune M, et al. Olfactory function and morphology of olfactory epithelium in an adult population with schizophrenia. Schizophr Res. 2014
12. Brewer WJ, Wood SJ, McGorry PD, et al. Impairment of olfactory identification ability in individuals at ultra-high risk for psychosis who later develop schizophrenia. Am J Psychiatry. 2003; 160:1790–1794. [PubMed: 14514492]
13. Ishizuka K, Tajinda K, Colantuoni C, et al. Negative symptoms of schizophrenia correlate with impairment on the University of Pennsylvania smell identification test. Neurosci Res. 2010; 66:106–110. [PubMed: 19819272]
14. Moberg PJ, Agrin R, Gur RE, et al. Olfactory dysfunction in schizophrenia: a qualitative and quantitative review. Neuropsychopharmacology. 1999; 21:325–340. [PubMed: 10457530]
15. English JA, Fan Y, Focking M, et al. Reduced protein synthesis in schizophrenia patient-derived olfactory cells. Transl Psychiatry. 2015; 5:e663. This discovery-based proteomics approach demonstrated that proteins involved in the eIF2/4 pathway and mTOR signaling were the most differentially expressed in olfactory neurosphere-derived cells between SZ and controls. A decreased expression of 17 ribosomal proteins was also observed in SZ patients, which was associated with a reduction in the rate of protein synthesis in SZ. These observations suggest an

alteration in protein expression regulation in olfactory neurosphere-derived cells from SZ patients, which may contribute to the aberrant neurodevelopmental processes of the disorder. [PubMed: 26485547]

16. Horiuchi Y, Kondo MA, Okada K, et al. Molecular signatures associated with cognitive deficits in schizophrenia: a study of biopsied olfactory neural epithelium. *Transl Psychiatry*. 2016; 6:e915. By using neuronal layers enriched by LCM from the OE, the SMAD pathway was found to be downregulated and potentially associated with cognitive deficits in SZ. [PubMed: 27727244]
17. Kano S, Colantuoni C, Han F, et al. Genome-wide profiling of multiple histone methylations in olfactory cells: further implications for cellular susceptibility to oxidative stress in schizophrenia. *Mol Psychiatry*. 2013; 18:740–742. [PubMed: 22925834]
18. Arnold SE, Han LY, Moberg PJ, et al. Dysregulation of olfactory receptor neuron lineage in schizophrenia. *Arch Gen Psychiatry*. 2001; 58:829–835. [PubMed: 11545665]
19. Borgmann-Winter K, Willard SL, Sinclair D, et al. Translational potential of olfactory mucosa for the study of neuropsychiatric illness. *Transl Psychiatry*. 2015; 5:e527. [PubMed: 25781226]
20. Mackay-Sim A. Stem cells and their niche in the adult olfactory mucosa. *Arch Ital Biol*. 2010; 148:47–58. [PubMed: 20830968]
21. Calof AL, Mumm JS, Rim PC, Shou J. The neuronal stem cell of the olfactory epithelium. *J Neurobiol*. 1998; 36:190–205. [PubMed: 9712304]
22. Mackay-Sima A, Chuahb MI. Neurotrophic factors in the primary olfactory pathway. *Prog Neurobiol*. 2000; 62:527–559. [PubMed: 10869782]
23. Hahn CG, Han LY, Rawson NE, et al. In vivo and in vitro neurogenesis in human olfactory epithelium. *J Comp Neurol*. 2005; 483:154–163. [PubMed: 15678478]
24. Chen CR, Kachramanoglou C, Li D, et al. Anatomy and cellular constituents of the human olfactory mucosa: a review. *J Neurol Surg B Skull Base*. 2014; 75:293–300. [PubMed: 25302141]
25. Schwob JE, Jang W, Holbrook EH, et al. Stem and progenitor cells of the mammalian olfactory epithelium: Taking poietic license. *J Comp Neurol*. 2016
26. Mackay-Sim A. Concise review: Patient-derived olfactory stem cells: new models for brain diseases. *Stem Cells*. 2012; 30:2361–2365. [PubMed: 22961669]
27. Marshall CT, Lu C, Winstead W, et al. The therapeutic potential of human olfactory-derived stem cells. *Histol Histopathol*. 2006; 21:633–643. [PubMed: 16528674]
28. Roisen FJ, Klueber KM, Lu CL, et al. Adult human olfactory stem cells. *Brain Res*. 2001; 890:11–22. [PubMed: 11164764]
29. Murrell W, Feron F, Wetzig A, et al. Multipotent stem cells from adult olfactory mucosa. *Dev Dyn*. 2005; 233:496–515. [PubMed: 15782416]
30. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*. 1992; 255:1707–1710. [PubMed: 1553558]
31. Ohnishi Y, Iwatsuki K, Ishihara M, et al. Isolation of human adult olfactory sphere cells as a cell source of neural progenitors. *Stem Cell Res*. 2015; 15:23–29. [PubMed: 25965912]
32. Murrell W, Wetzig A, Donnellan M, et al. Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease. *Stem Cells*. 2008; 26:2183–2192. [PubMed: 18535154]
33. Wetzig A, Mackay-Sim A, Murrell W. Characterization of olfactory stem cells. *Cell Transplant*. 2011; 20:1673–1691. [PubMed: 21535908]
34. Matigian N, Abrahamsen G, Sutharsan R, et al. Disease-specific, neurosphere-derived cells as models for brain disorders. *Dis Model Mech*. 2010; 3:785–798. [PubMed: 20699480]
35. Fan Y, Abrahamsen G, McGrath JJ, Mackay-Sim A. Altered cell cycle dynamics in schizophrenia. *Biol Psychiatry*. 2012; 71:129–135. [PubMed: 22074612]
36. Fan Y, Abrahamsen G, Mills R, et al. Focal adhesion dynamics are altered in schizophrenia. *Biol Psychiatry*. 2013; 74:418–426. [PubMed: 23482246]
37. Delorme B, Nivet E, Gaillard J, et al. The human nose harbors a niche of olfactory ectomesenchymal stem cells displaying neurogenic and osteogenic properties. *Stem Cells Dev*. 2010; 19:853–866. [PubMed: 19905894]
38. Zhang X, Klueber KM, Guo Z, et al. Induction of neuronal differentiation of adult human olfactory neuroepithelial-derived progenitors. *Brain Res*. 2006; 1073-1074:109–119. [PubMed: 16455065]

39. Benitez-King G, Riquelme A, Ortiz-Lopez L, et al. A non-invasive method to isolate the neuronal lineage from the nasal epithelium from schizophrenic and bipolar diseases. *J Neurosci Methods*. 2011; 201:35–45. [PubMed: 21787803]
40. Brown AS, Borgmann-Winter K, Hahn CG, et al. Increased Stability of Microtubules in Cultured Olfactory Neuroepithelial Cells from Individuals with Schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013
41. Feron F, Perry C, Hirning MH, et al. Altered adhesion, proliferation and death in neural cultures from adults with schizophrenia. *Schizophr Res*. 1999; 40:211–218. [PubMed: 10638859]
42. Horiuchi Y, Kano S, Ishizuka K, et al. Olfactory cells via nasal biopsy reflect the developing brain in gene expression profiles: utility and limitation of the surrogate tissues in research for brain disorders. *Neurosci Res*. 2013; 77:247–250. [PubMed: 24120685]
43. Tajinda K, Ishizuka K, Colantuoni C, et al. Neuronal biomarkers from patients with mental illnesses: a novel method through nasal biopsy combined with laser-captured microdissection. *Mol Psychiatry*. 2010; 15:231–232. [PubMed: 20168323]
44. Narayan S, McLean C, Sawa A, et al. Olfactory neurons obtained through nasal biopsy combined with laser-capture microdissection: a potential approach to study treatment response in mental disorders. *J Vis Exp*. 2014
45. Brennand K, Savas JN, Kim Y, et al. Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. *Mol Psychiatry*. 2015; 20:361–368. [PubMed: 24686136]
46. Mor E, Kano S, Colantuoni C, et al. MicroRNA-382 expression is elevated in the olfactory neuroepithelium of schizophrenia patients. *Neurobiol Dis*. 2013; 55:1–10. [PubMed: 23542694]
47. Solis-Chagoyan H, Calixto E, Figueroa A, et al. Microtubule organization and L-type voltage-activated calcium current in olfactory neuronal cells obtained from patients with schizophrenia and bipolar disorder. *Schizophr Res*. 2013; 143:384–389. [PubMed: 23290267]
48. McCurdy RD, Feron F, Perry C, et al. Cell cycle alterations in biopsied olfactory neuroepithelium in schizophrenia and bipolar I disorder using cell culture and gene expression analyses. *Schizophr Res*. 2006; 82:163–173. [PubMed: 16406496]
49. Pantazopoulos H, Boyer-Boiteau A, Holbrook EH, et al. Proteoglycan abnormalities in olfactory epithelium tissue from subjects diagnosed with schizophrenia. *Schizophr Res*. 2013; 150:366–372. [PubMed: 24035561]
50. Feron F, Gepner B, Lacassagne E, et al. Olfactory stem cells reveal MOCOS as a new player in autism spectrum disorders. *Mol Psychiatry*. 2015 At the molecular level, it was found that MOCOS, involved in purine metabolism, was downregulated in nasal olfactory stem cells isolated from nasal biopsies of adults with autism.
51. Ronnett GV, Leopold D, Cai X, et al. Olfactory biopsies demonstrate a defect in neuronal development in Rett's syndrome. *Ann Neurol*. 2003; 54:206–218. [PubMed: 12891673]
52. Abrams MT, Kaufmann WE, Rousseau F, et al. FMR1 gene expression in olfactory neuroblasts from two males with fragile X syndrome. *Am J Med Genet*. 1999; 82:25–30. [PubMed: 9916838]
53. Hahn CG, Gomez G, Restrepo D, et al. Aberrant intracellular calcium signaling in olfactory neurons from patients with bipolar disorder. *Am J Psychiatry*. 2005; 162:616–618. [PubMed: 15741484]
54. Arnold SE, Lee EB, Moberg PJ, et al. Olfactory epithelium amyloid-beta and paired helical filament-tau pathology in Alzheimer disease. *Ann Neurol*. 2010; 67:462–469. [PubMed: 20437581]
55. Talamo BR, Rudel R, Kosik KS, et al. Pathological changes in olfactory neurons in patients with Alzheimer's disease. *Nature*. 1989; 337:736–739. [PubMed: 2465496]
56. Tabaton M, Cammarata S, Mancardi GL, et al. Abnormal tau-reactive filaments in olfactory mucosa in biopsy specimens of patients with probable Alzheimer's disease. *Neurology*. 1991; 41:391–394. [PubMed: 1900925]
57. Wolozin B, Lesch P, Lebovics R, Sunderland TAE. Bennett Research Award 1993. Olfactory neuroblasts from Alzheimer donors: studies on APP processing and cell regulation. *Biol Psychiatry*. 1993; 34:824–838. [PubMed: 8110910]

58. Kulkarni-Narla A, Getchell TV, Schmitt FA, Getchell ML. Manganese and copper-zinc superoxide dismutases in the human olfactory mucosa: increased immunoreactivity in Alzheimer's disease. *Exp Neurol*. 1996; 140:115–125. [PubMed: 8690055]
59. Getchell ML, Shah DS, Buch SK, et al. 3-Nitrotyrosine immunoreactivity in olfactory receptor neurons of patients with Alzheimer's disease: implications for impaired odor sensitivity. *Neurobiol Aging*. 2003; 24:663–673. [PubMed: 12885574]
60. Ghanbari HA, Ghanbari K, Harris PL, et al. Oxidative damage in cultured human olfactory neurons from Alzheimer's disease patients. *Aging Cell*. 2004; 3:41–44. [PubMed: 14965355]
61. Cook AL, Vitale AM, Ravishankar S, et al. NRF2 activation restores disease related metabolic deficiencies in olfactory neurosphere-derived cells from patients with sporadic Parkinson's disease. *PLoS One*. 2011; 6:e21907. [PubMed: 21747966]

Key points

- The olfactory epithelium (OE) retains the capacity for lifelong neurogenesis and regeneration due to the presence of neural stem cells and progenitors.
- Thus, there is no need for genetic reprogramming to obtain neural progenitors and mature neurons from the OE.
- Accordingly, OE-derived cell/tissue models have been used as a surrogate system to study the molecular mechanisms implicated in schizophrenia (SZ).
- By using OE-derived tissue/cell models, alterations in neurodevelopment, stress response, and gene/protein expression regulatory pathways have been recently found in patients with SZ.

Figure 1. The human olfactory epithelium with neural progenitors

The olfactory neural epithelium is composed of different cell types including basal cells (prognitor cells), olfactory neurons (mature and immature), and sustentacular cells. Adapted from Sawa A and Cascella NG, *Am J Psychiatry*, 2009, 166(2):137-139.

Table 1
Findings from olfactory epithelium-derived tissue/cell models obtained from patients with SZ and other brain disorders

Disorder	Model type		Main findings from patients	Reference
Schizophrenia	Olfactory neurospheres and their derived cells		Disease-specific alterations in gene expression, protein expression, and cell function, including dysregulated neurodevelopmental pathways.	Matigian N et al. Disease Models and Mechanisms, 2010
			Reduction in protein synthesis rate. Dysregulation of proteomic and transcriptomic signatures converging toward perturbation of the eukaryotic translation initiation factor 2A (eIF2a), eIF4, and mechanistic target of rapamycin (mTOR) pathways.	English JA et al. Translational Psychiatry, 2015
			Increase in proliferation rate. Shorter cell cycle period. Higher levels of cyclins D1, E, and A2.	Fan Y et al. Biological Psychiatry, 2012
			Altered motility and focal adhesion dynamics. Dysregulated gene expression in the focal adhesion kinase signalling pathway.	Fan Y et al. Biological Psychiatry, 2013
	Non-neurosphere approach	Culture	Increased miRNA-382 expression in cultured olfactory cells and in olfactory neuronal layer tissues enriched by laser captured microdissection.	Mor E et al. Neurobiology of Disease, 2013
			Alterations in H3K4me3 and/or H3K27me3 levels affecting the expression of genes involved in cellular protection against oxidative stress.	Kano S et al. Molecular Psychiatry, 2013
			Extensive, unstained subcellular areas with the anti- β -tubulin III antibody and disorganized microtubules. Decrease in L-type calcium current.	Solis-Chagoyan H et al. Schizophrenia Research, 2013
			Increased stability of microtubules. Greater percentage of cells with intact microtubules.	Brown AS et al. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 2014
			Reduced ability to attach to the culture slide. Greater proportion of cells undergoing mitosis.	Feron F et al. Schizophrenia Research 1999
			Higher ratio of mitotic cells. Differential expression of genes related to cell cycle and RNA metabolism.	McCurdy RD et al. Schizophrenia Research, 2006
		Tissue	Downregulation of the SMAD pathway that may be associated with cognitive deficits.	Horiuchi Y, Translational Psychiatry, 2016
			Decrease in the density of p75-nerve growth factor receptor (NGFR)-positive basal cells, which indicates dysregulation in olfactory receptor neuronal development.	Minovi A et al. Schizophrenia Research, 2015
			Abnormal densities and ratios of olfactory epithelium neurons at different stages of development, which may indicate a dysregulation	Arnold SE et al. Arch Gen Psychiatry, 2001

Disorder	Model type		Main findings from patients	Reference
			of olfactory epithelium neuronal lineage.	
			Reduction of chondroitin sulfate proteoglycans (CSPGs) in mature olfactory receptor neurons.	Pantazopoulos H et al. Schizophrenia Research, 2013
Autism	Non-neurosphere approach	Culture	Downregulation of molybdenum cofactor sulfurase (MOCOS) at the RNA and protein levels.	Feron F et al. Mol Psychiatry, 2016
Rett Syndrome	Non-neurosphere approach	Tissue	Dysmorphic neurons. Increased ratio of immature to mature neurons.	Ronnett GV et al. Annals of Neurology, 2003
Fragile X Syndrome	Non-neurosphere approach	Culture	Demonstrated feasibility of using olfactory neuroblasts to evaluate the fragile X mental retardation 1 gene (<i>FMR1</i>) mutation in humans <i>in vivo</i> .	Abrams MT et al. American Journal of Medical Genetics, 1999
Bipolar Disorder	Non-neurosphere approach	Dissociated olfactory receptor neurons	Decrease in intracellular calcium.	Hahn CG et al. American Journal of Psychiatry, 2005
		Culture	Shorter microtubules.	Solis-Chagoyan H et al. Schizophrenia Research 2013
		Tissue	Higher level of glycogen synthase kinase-3 beta (GSK3b) that is reduced by lithium treatment.	Narayan S et al. Journal of Visual Experiments, 2014
Alzheimer's Disease	Non-neurosphere approach	Culture	Elevated levels of the amyloid precursor protein C-terminal derivative (CTD), which is the direct precursor to beta-amyloid.	Wolozin B et al. Biological Psychiatry, 1993
			Increased levels of the oxidative stress markers, heme oxygenase-1, hydroxynonenal pyrrole, and N-carboxymethyl lysine.	Ghanbari HA et al. Aging Cell, 2004
		Tissue	Increased neurofilament immunoreactivity and abnormal neuronal structure that are correlated with brain pathology.	Talamo BR et al. Nature, 1989
			Presence of tau- and ubiquitin-reactive dystrophic neurites in the lamina propria.	Tabaton M et al. Neurology, 1991
			Increased immunoreactivity for manganese and copper-zinc superoxide dismutase.	Kulkarni-Narla A et al. Experimental Neurology, 1996
			Increased immunoreactivity for 3-nitrotyrosine in olfactory receptor neurons and CD68 in macrophages.	Getchell ML et al. Neurobiology of Aging, 2003
			Expression of beta-amyloid and paired helical filament-tau in olfactory epithelium significantly correlated with brain pathology.	Arnold SE et al. Annals of Neurology, 2010
Parkinson's Disease	Olfactory neurospheres and their derived cells		Disease-specific alterations in gene and protein expression, including dysregulation of those involved in mitochondrial function, oxidative stress, and xenobiotic metabolism.	Matigian N et al. Disease Models and Mechanisms, 2010
			Reduction in glutathione levels and MTS metabolism that is restored by activation of the NRF2 pathway.	Cook AL et al. PLoS ONE, 2011