Trends in Molecular Medicine

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Forum

The Olfactory Neural Epithelium As a Tool in Neuroscience

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Capturing both dynamic changes (state) and persistent signatures (trait) directly associated with disease at the molecular level is crucial in modern medicine. The olfactory neural epithelium, easily accessible in clinical settings, is a promising surrogate model in translational brain medicine, complementing the limitations in current engineered cell models.

Investigating molecular biomarkers and mechanisms of brain disorders has been a significant challenge due to the difficulty in obtaining tissues and cells from the central nervous system (CNS) of living patients. As a result, research for brain disorders has used a wide range of models including animal models, postmortem brains, and peripheral cells derived from living patients to understand the pathological mechanisms of disease. Blood samples may be useful as high-throughput resources, but they may not represent neuron-associated molecular signatures. Postmortem brains are valuable resources for studying molecular signatures within the complex neural architectures, but do not reliably provide any molecular information associated with the onset or the course of functional impairments, including the dynamic 'state' changes in living patients. Hence, although such samples have contributed significantly to studies of geriatric neurodegenerative disorders, their contribution to an understanding of neurodevelopmental diseases has been more limited.

Recently, cell modeling utilizing reprogramming procedures has been developed to culture human neural cells from living patients *in vitro*, including induced neuronal (iN) cells and induced pluripotent stem cells (iPSCs)-derived neurons. These engineered cell models may reflect more accurate neural 'traits' of brain cells, but molecular and cellular signatures at the time of a biopsy ('state' changes) may be lost in the process. Despite being highly informative, these models harbor some limitations that hamper the study of brain disorder pathophysiology [1].

The potential of using the olfactory neural epithelium (OE) (Box 1) as a surrogate model to study the CNS was examined decades ago [2]. However, the purity of neural cells from biopsied tissues was suboptimal. Although the procedure of nasal biopsy is relatively noninvasive and almost equivalent to a skin-punch biopsy, the efforts to make this process more efficient and even less invasive are emerging.

With recent technical advances overcoming past drawbacks, here, we reintroduce the discussion of using the OE as a useful surrogate model to investigate brain disorders, complementing iPSCs approaches. We argue that using a combination of these models may represent an important strategy to advance our understanding of brain diseases.

Advances in Sample Preparation

Major advances in the use of the OE for translational research include the exploration of less-invasive approaches to obtain tissue biopsies as well as increased efforts to enrich and purify neural cells from biopsied tissues.

A Less-Invasive Biopsy Approach: The Brush Swab

OE tissue was conventionally obtained by punch biopsy through nasal endoscopy [3]. By contrast, a newly developed brushing technique allows a quicker and less-invasive biopsy minimizing or avoiding the use of local anesthesia. A cytology brush is placed in the nasal cavity, and gently rotated to collect epithelial cells. Specimens can be immediately placed in culture media and processed. The exfoliated cells in culture can be propagated to establish neural precursor banks exhibiting cytoskeletal phenotypes of developing neurons [4].

Enriching Neural Cells: Laser-Captured Microdissection and Olfactory Neurospheres

Recently, two major efforts in enriching neural cells from biopsied OE have been undertaken. First, by combining nasal biopsies with laser-captured microdissection (LCM), the neural layer can be selectively isolated from the OE. The use of this technique dramatically increases the relative expression of olfactory marker protein – a marker for mature neurons. By contrast, the relative level of aldehyde dehydrogenase 1A3, a nasal submucosal marker, is negligible in microdissected tissue relative to the undissected one [5]. Given that one of the major challenges in neuroscience is identifying 'state'-dependent neural molecular changes, this protocol provides an opportunity to capture molecular snapshots of neural tissue at the time of biopsy. In addition, fresh cells collected from OE by brush swab can be utilized in the future as resources for studying 'state' molecular alterations associated with brain disorders in combination with single-cell profiling technologies (Table 1).

Second, an advantage of using human OE is that it may be possible to obtain stem cells from the olfactory mucosa, propagated as progenitor cells in olfactory neurospheres [6]. These cells can be maintained in culture and differentiated

Box 1. The Olfactory Neural Epithelium

The olfactory neural epithelium (OE) is located in the most superficial layer of the olfactory mucosa containing different cell types, including olfactory neurons, olfactory ensheathing cells, sustentacular cells, as well as the horizontal and globose basal cells. Basal cells are considered to be multipotent and/or neural precursor cells that can proliferate and differentiate into either neural or non-neural cells in both humans and rodents. Mature and immature (transitional) olfactory neurons are located in the intermediate layer of the OE, while the apical layer contains sustentacular cells and sensory cilia that are projected from the dendrites of olfactory neurons. The histological scheme is shown in Figure I.

Olfactory neurons are continuously replaced by neurogenesis in the OE throughout adult life. This process is regulated by growth factors that also control neurogenesis in the CNS. An extensive resource about the anatomy and the cellular markers of the olfactory biopsied tissue has been previously published [15].

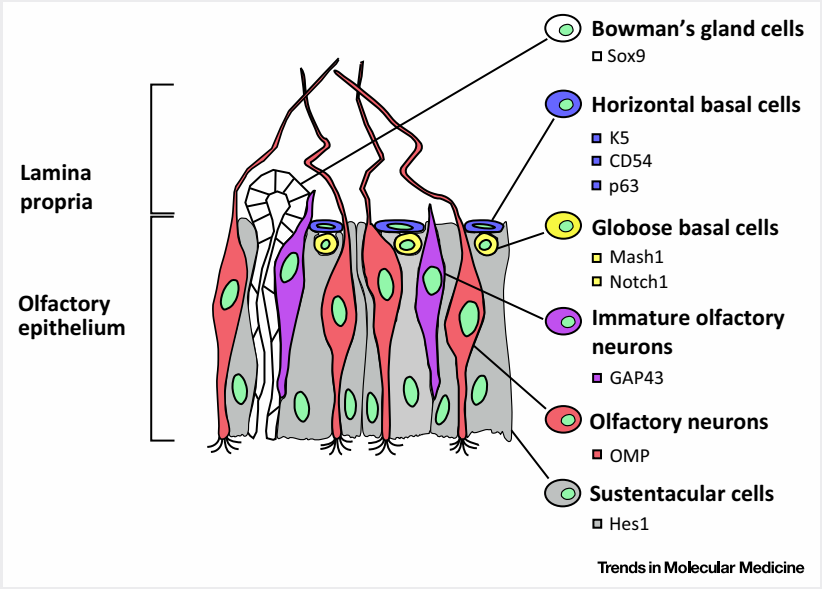

Figure I. The Human Olfactory Epithelium with Specific Markers for Different Cell Types. The olfactory neural epithelium is composed of different cell types including Bowman's gland cells, horizontal basal cells, globose basal cells, olfactory neurons (mature and immature), and sustentacular cells. These cell types express specific markers of differentiation, as represented in the anatomical scheme. Abbreviations: CD54, cluster of differentiation 54; GAP43, growth-associated protein 43; Hes1, hairy and enhancer of split-1; K5, keratin 5; Mash1, mammalian achaete-scute homolog-1; Notch1, neurogenic locus notch homolog protein 1; OMP, olfactory marker protein; p63, tumor protein p63; Sox9, Sry-related HMG box 9. Adapted from [16].

into several types of cells such as neurons and glia. While significant disease-specific alterations in the genome and proteome have been reported in olfactory neurosphere-derived cells of patients with schizophrenia, fibroblasts from these patients have failed to show any disease-specific alterations [6], supporting the utility of OE cells in disease models.

Furthermore, OE tissues are currently being processed in other methodologies for translational efforts such as the investigation of 'trait' signatures through biochemical and molecular studies. Cultured OE cells have revealed similar gene expression profiles than stem cells and brain tissues, but not blood cells [7]. OE can also be sectioned, fixed, and used for histochemistry purposes; indeed, similar histochemical signatures have been reported between olfactory neurons from biopsied OE and autopsied cerebral tissues from Alzheimer's disease patients [8].

Pros and Cons: What Is Needed in the System?

By properly addressing unanswered questions on this methodology, it may be eventually possible to control its limitations and fine-tune appropriate experimental designs combining different cell models.

Capturing 'State' Changes Directly

Using whole OE tissue, LCM-enriched neural tissue, and the brush-swabbed cells, it is possible to investigate 'state' markers at the time of biopsy. This may represent an important advantage relative to other models such as iPSCs-derived neurons, where such 'state' markers are likely to be 'erased' during the course of cell reprogramming and maintenance. For example, OE biopsies combined with LCM have been used to establish a platform to detect neural molecular changes before and after lithium treatment in patients with bipolar disorder [9]. This suggests that, by using this system, it might be possible to investigate molecular 'state' changes in response to specific pharmacological treatments, and/or directly associated with brain disease.

No Genetic Reprogramming

A valuable advantage of using OE-derived cells is that they are free from epigenetic changes occurring following a reprogramming procedure – a valid concern in various engineered cell models. Epigenetic changes can occur during the reprogramming process, which possibly interferes with an accurate assessment of differential gene expression and epigenetic programs in cells associated with brain disorders [10]. In addition, the procedures involved in generating iPSCs and deriving neurons from these are very time-consuming, costly, and are not high-throughput. By using OE-derived neurons, confounding events during genetic reprogramming can be bypassed. Thus, we suggest that combining findings from both OE-derived neurons and iPSCs-derived neurons might constitute a powerful complementary strategy to