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Review

The microtubular cytoskeleton of olfactory neurons derived from patients with schizophrenia or with bipolar disorder: Implications for biomarker characterization, neuronal physiology and pharmacological screening

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ABSTRACT

Schizophrenia (SZ) and Bipolar Disorder (BD) are highly inheritable chronic mental disorders with a worldwide prevalence of around 1%. Despite that many efforts had been made to characterize biomarkers in order to allow for biological testing for their diagnoses, these disorders are currently detected and classified only by clinical appraisal based on the Diagnostic and Statistical Manual of Mental Disorders. Olfactory neuroepithelium-derived neuronal precursors have been recently proposed as a model for biomarker characterization. Because of their peripheral localization, they are amenable to collection and suitable for being cultured and propagated *in vitro*. Olfactory neuroepithelial cells can be obtained by a non-invasive brush-exfoliation technique from neuropsychiatric patients and healthy subjects. Neuronal precursors isolated from these samples undergo *in vitro* the cytoskeletal reorganization inherent to the neurodevelopment process which has been described as one important feature in the etiology of both diseases. In this paper, we will review the current knowledge on microtubular organization in olfactory neurons of patients with SZ and with BD that may constitute specific cytoskeletal endophenotypes and their relation with alterations in L-type voltage-activated Ca^{2+} currents. Finally, the potential usefulness of neuronal precursors for pharmacological screening will be discussed.

Contents

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1. Introduction

Schizophrenia (SZ) and Bipolar Disorder (BD) are highly heritable chronic mental disorders with a worldwide prevalence of around 1%. SZ is characterized by abnormalities in reality perception and expression and is manifested by hallucinations, delusions, and disorganized speech and thinking, while BD is characterized by alternating episodes of severe depression and mania and alterations in sleep profiles (hypersomnia or insomnia), energy, thinking, and behavior. Although known as clinically different entities, these diseases have genetic and developmental risk factors in common, as well as neuropsychological profiles. In addition, both diseases share symptoms such as motor impairments called the neurological soft signs (NSS) and neurocognitive impairment, as well as structural brain abnormalities such as gray matter reduction, diminished volume in the hippocampus, enlargement of the ventricular zone and synaptic dysconnectivity associated with psychosis (Beasley et al., 2009; Chan et al., 2013; Chrobak et al., 2016; Hajek et al., 2005; Kumar et al., 2015; Murray et al., 1991; Rosoklija et al., 2000; Wang et al., 2010; Yuksel et al., 2012).

In recent years, the olfactory neuroepithelium has been used as a model to investigate the neurodevelopment process related with the etiology of SZ and BD (Cascella et al., 2007; Feron et al., 1999; Hahn et al., 2005a). This tissue is continuously regenerated throughout life. It is enriched in stem cells, which are differentiated into immature neurons that migrate to the apical side of the neuroepithelium to produce mature olfactory neurons (Calof and Chikaraishi, 1989; Cascella et al., 2007; DeHamer et al., 1994; Mackay-Sim and Kittel, 1991). Due to its peripheral localization, it is amenable to collection, therefore suitable for being cultured and propagated *in vitro*.

The technique to obtain biopsy specimens of human olfactory mucosa was developed in 1982 (Lovell et al., 1982). Years later, olfactory neuronal precursors from adult human cadavers were obtained and cultured by Wolozin et al. (1992). Since that decade many groups have developed methods for the isolation and culture of olfactory neuronal precursors from biopsies of human cadavers or living neuropsychiatric patients (Feron et al., 1998, 2013; Girard et al., 2011; Lanza et al., 1993; Murrell et al., 2008; Narayan et al., 2014). Recently, an exfoliation procedure using a brushing technique to collect neuronal precursors and stem cells from the neuroepithelium was developed. This procedure is non-invasive and has been applied to healthy subjects and psychiatric patients to obtain cells of neuronal lineage (Benítez-King et al., 2011).

In this review, we will describe the main components of the neuroepithelial tissue and the evidence that supports the usefulness of biopsies in the study of the neurodevelopment process in relation to the etiology of SZ and BD as a mirror model of the Central Nervous System (CNS) neurons. We will also discuss the additional advantages of the study of cultured neuronal precursors in cell monolayers for biomarker characterization and evaluation of pharmacological responses (theranostics). In addition, we will review the evidence supporting that patient-derived olfactory neuronal precursors display differential cytoskeleton endophenotypes together with differential alterations in L-type voltage-activated Ca^{2+} currents.

2. The olfactory neuroepithelium

The olfactory neuroepithelium is a pseudostratified columnar epithelium specialized in odor perception. This tissue is located in the nasal cavity at the cribiform plate and at the superior and middle turbinate region intercalated with respiratory epithelial patches (Jafek et al., 1997). It is constituted of stem cells (globose and horizontal cells), neuronal precursors, Bowman's glands, olfactory sensory neurons, and glia-like supporting cells: the sustentacular cells. The apical side of the olfactory neuroepithelium is in close contact with the environment and is the most external layer of the nasal cavity. Cilia of olfactory neurons are also localized in the apical layer of the neuroepithelium and are projected into the nasal cavity, exposing odor receptors that bind odoriferous substances present in the environment (Hempstead and Morgan, 1983; Monahan and Lomvardas, 2015). The middle layer of the olfactory neuroepithelium is constituted by the somas of the olfactory sensory neurons, which transmit odor information to the olfactory bulb (Calof and Chikaraishi, 1989). The third layer of the neuroepithelial tissue is located in close contact with the basal lamina and contains both horizontal basal cells and globose cells (Graziadei and Graziadei, 1979). Both of these cell types have been described as multipotent stem cells and are considered highly neurogenic because they actively proliferate and give rise to sustentacular cells and olfactory neurons that replace neuroepithelia damaged by the environment (Calof et al., 1998; Chen et al., 2014).

3. Olfactory neurons as a mirror model of the central nervous system

The difficulty in studying biomarkers in mental illnesses, as well as the elucidation of their etiology, lies in the ethical reasons for the unavailability of neuronal cells obtained from the human CNS. Therefore, the majority of research has been performed in peripheral tissues, which have the inconvenience of deriving from non-neuronal lineages (Mamdani et al., 2013). Cells from peripheral blood can be easily collected and express some candidate genes for SZ; however, their gene expression profile is very distant from that of neurons (Hahn et al., 2005b; Sullivan et al., 2006). In addition, important information has been gathered from human brain tissues obtained postmortem. However, deterioration of the sample by the postmortem interval, as well as factors such as medication, drug abuse, and lifestyle, among others, cannot be ruled out (Ferrer et al., 2008; Lipska et al., 2006).

In the previous decade, there was emerging interest in studying olfactory neuroepithelial biopsies to validate the neurodevelopmental hypothesis of SZ (Cascella et al., 2007; Feron et al., 1998, 1999; McCurdy et al., 2006). In fact, olfactory neurons are closer to CNS neurons than any other peripheral cells used to characterize biomarkers. These neurons have a common ectodermal embryonic origin with CNS neurons, and derive from embryonic placodes and the neural crest, which are structures analogous to the neural tube (Forni and Wray, 2012; Sidman and Rakic, 1973). In addition, gene expression profiles of olfactory cells are close to mesenchymal stem cells that can be differentiated into neurons (Delorme et al., 2010; Horiuchi et al., 2013). Expression of hundreds of genes similar to CNS neurons has been described in olfactory neuroepithelial cells, such as the pituitary adenylate cyclase-activating peptide and the glutamate receptor, among others (Hegg et al., 2003; Kato et al., 2007; Matigian et al., 2010; Thukral et al., 1997). Moreover, in postmortem samples from Alzheimer's disease patients, paired helical filaments of tau protein and amyloid-β plaques similar to those found in cortical and subcortical neurons, have been described in olfactory neuroepithelial cells characterized by cytokeratin-18 expression reflecting its stromal epithelial cell nature, as well as in olfactory neurons characterized by III β-tubulin expression (Arnold et al., 2010; Ayala-Grosso et al., 2015). This supports that olfactory neuroepithelial cells are a mirror model that reflects molecular changes produced in the CNS.

Additionally, patients with SZ and with BD display alterations in the olfactory system, such as diminished olfactory bulb volume and impairments in odor detection and odor discrimination (Cumming et al., 2011; Lovdahl et al., 2014; Moberg et al., 2006; Nguyen et al., 2010; Takahashi et al., 2014; Turetsky et al., 2009). Moreover, the olfactory neural pathway is closely associated with frontal and temporal brain regions, which are structures known to play a key role in the etiology of SZ and BD (Ellison-Wright and Bullmore, 2010). Taken together, the evidence supports that the olfactory neuroepithelium comprises an experimental mirror model that reflects transcriptional as well as biochemical, structural, and metabolic changes of CNS neurons. Therefore, this tissue may be useful for biomarker characterization at the structural