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Differentiation of oligodendrocyte precursors is impaired in the prefrontal cortex in schizophrenia

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ABSTRACT

The pathophysiology of schizophrenia involves disturbances of information processing across brain regions, possibly reflecting, at least in part, a disruption in the underlying axonal connectivity. This disruption is thought to be a consequence of the pathology of myelin ensheathment, the integrity of which is tightly regulated by oligodendrocytes. In order to gain insight into the possible neurobiological mechanisms of myelin deficit, we determined the messenger RNA (mRNA) expression profile of laser captured cells that were immunoreactive for 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), a marker for oligodendrocyte progenitor cells (OPCs) in addition to differentiating and myelinating oligodendrocytes, in the white matter of the prefrontal cortex in schizophrenia subjects. Our findings pointed to the hypothesis that OPC differentiation might be impaired in schizophrenia. To address this hypothesis, we quantified cells that were immunoreactive for neural/glial antigen 2 (NG2), a selective marker for OPCs, and those that were immunoreactive for oligodendrocyte transcription factor 2 (OLIG2), an oligodendrocyte lineage marker that is expressed by OPCs and maturing oligodendrocytes. We found that the density of NG2-immunoreactive cells was unaltered, but the density of OLIG2-immunoreactive cells was significantly decreased in subjects with schizophrenia, consistent with the notion that OPC differentiation impairment may contribute to oligodendrocyte disturbances and thereby myelin deficits in schizophrenia.

1. Introduction

Schizophrenia can be conceptualized as the clinical manifestation of the dysfunction of information processing and integration of neural networks across brain regions, including the different areas of the cerebral cortex and subcortical domains, such as the thalamus and medial temporal structures. It is well established that neuronal circuits within these regions are functionally disturbed in schizophrenia. Furthermore, impaired myelin ensheathment of the axons that connect these neuronal circuits via the white matter can further exacerbate network dysfunction by disturbing the timing of the arrival of action potential, compromising spike-timing dependent potentiation and hence disrupting the precise temporal coherence of neuronal circuit output that is necessary for synchronized network activation (Fields, 2005; Fields et al., 2014; Pajevic et al., 2013) which, in turn, may underlie many of the cognitive and perceptual deficits that are characteristic of schizophrenia (Cho et al., 2006; Lisman and Buzsaki, 2008; Spencer et al., 2004; Uhlhaas et al., 2008; Uhlhaas and Singer, 2010).

Structural magnetic resonance imaging (MRI) investigations have converged upon the conclusion that the volume of the white matter is decreased in patients with schizophrenia (Bora et al., 2011; Breier et al., 1992; Buchanan et al., 1998; Sigmundsson et al., 2001), consistent with the notion that the integrity of myelin is compromised. Furthermore, diffusion tensor imaging (DTI) has revealed that fractional anisotropy, which is a measure of a combination of factors, including myelin sheath integrity, is also decreased in schizophrenia patients (Holleran et al., 2014). More direct evidence of myelin deficit in this illness has come from postmortem studies. Specifically, the density of oligodendrocytes has been found to be decreased by 25–31% in both the gray and white matter of the prefrontal cortex in schizophrenia subjects (Hof et al., 2003; Uranova et al., 2004, 2007; Vostrikov and Uranova, 2011). Furthermore, ultrastructural examination of oligodendrocytes has revealed morphological changes associated with cell degeneration and death (Uranova et al., 2001, 2007), in addition to disturbances in the integrity of myelinated axonal fibers (Uranova et al., 2011). Finally, gene expression profiling of RNA extracted from homogenized gray and white matter of the cerebral cortex have revealed that many oligodendrocyte- and myelin-associated genes appear to be differentially expressed in subjects with schizophrenia (Aston et al., 2004; Hakak et al., 2001; Haroutunian et al., 2007; Hof et al., 2002; Katsel et al.,

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2005; Mitkus et al., 2008; Sequeira et al., 2012; Sugai et al., 2004; Tkachev et al., 2003).

Myelin is actively maintained by a complex series of events that tightly regulate the generation, differentiation, survival and death of oligodendrocytes. In the adult rat brain, roughly 5–10% of cells are oligodendrocyte progenitors (OPCs) (Dawson, 2003; Polito and Reynolds, 2005), whereas approximately 20% of the cells in the white matter in humans may be OPCs (Lojewski et al., 2014). It is further estimated that up to 80% of OPCs are actively replicating or differentiating (Trotter et al., 2010; Young et al., 2013). Disturbances of any of the events that underlie the differentiation and mediate the integrity of oligodendrocytes could lead to myelin and myelination deficits.

In an attempt to gain insight into the possible molecular mechanisms specifically associated with the dysfunction of cells that belong to the oligodendrocyte lineage and thereby myelin and myelination deficits in the white matter, we profiled the expression of mRNA in these cells obtained by laser-capture microdissection (LCM). CNPase (2′,3′-cyclic nucleotide 3′-phosphodiesterase) was chosen as a marker, because it is expressed in OPCs in addition to differentiating and myelinating oligodendrocytes (Belachew et al., 2001; Dawson, 2003; Levine et al., 1993; Polito and Reynolds, 2005; Reynolds et al., 2002; Scherer et al., 1994; Sprinkle, 1989; Tomassy and Fossati, 2014). Together with immunohistochemical validation of findings of our gene expression profiling experiment using oligodendrocyte lineage markers including neural/glial antigen 2 (NG2), a marker for OPCs, and oligodendrocyte transcription factor 2 (OLIG2), which is present in maturing and mature, myelinating oligodendrocytes, the preponderance of evidence derived from the present study appears to favor the interpretation that impairment in the differentiation of OPCs may contribute to the pathophysiology of white matter deficits of schizophrenia.

2. Materials and methods

2.1. Postmortem human brain tissue

Fresh-frozen tissue blocks containing the prefrontal cortex (Brodmann's area 9) from 9 schizophrenia and 9 normal control subjects, matched for age, sex and postmortem interval (PMI), were obtained from the Harvard Brain Tissue Resource Center (Table 1). A detailed methodology for tissue preparation, LCM and RNA processing has been described in detail elsewhere (Pietersen et al., 2009, 2011, 2014a,b; Simunovic et al., 2009). Postmortem human brain collection procedures have been approved by the Partners Human Research Committee. Written informed consent for use of each of the brains for research has been obtained by the legal next-of-kin. The diagnosis of schizophrenia was made by two Board-certified psychiatrists by reviewing medical records and an extensive family questionnaire that included medical, psychiatric and social history. All of the brains included in this study were also examined by a Board-certified neuropathologist to rule out any neurological conditions. In addition, none of the subjects had any history of active substance abuse or dependence, as confirmed by toxicological analysis.

Table 1
Cases used in the present study.

Diagnosis	Age	Sex	PMI	aRNA concentration ng/μl
Schizophrenia	69	F	23.08	923.13
Schizophrenia	75	F	18.66	457.45
Schizophrenia	58	M	25.33	519.9
Schizophrenia	77	M	25.33	1159.7
Schizophrenia	66	M	21.75	845.9
Schizophrenia	63	M	26.16	450.07
Schizophrenia	60	F	17.13	706.1
Schizophrenia	60	M	22.17	1091.2
Schizophrenia	55	F	22	772.6
	64.8 ± 7.6		22.4 ± 3.0	769.6 ± 262.4
Control	69	F	18.65	1700
Control	78	F	18.7	295.16
Control	58	M	20.75	665.7
Control	76	M	23.92	684.6
Control	58	M	19.28	1437.88
Control	63	M	17.92	968.2
Control	60	F	21.67	645
Control	61	M	21	644.6
Control	63	F	23.5	800.4
	65.1 ± 8.4		20.1 ± 2.1	871.3 ± 437.9

2.2. Laser-capture microdissection

Sections of 8 μm were cut on a cryostat, mounted on slides and stored at −80 °C until use. Cells immunoreactive for CNPase, a myelin-associated enzyme that recognizes OPCs and both mature and immature oligodendrocytes, from the white matter (within 50 μm from the gray matter border) in the PFC were isolated using the Arcturus XT™ system (Life Technologies, Grand Island, CA). Approximately 400 cells per subject were captured onto a CapSure HS™ LCM cap (Life Technologies, Grand Island, CA). To avoid systematic biases, samples from schizophrenia and normal control subjects were processed for LCM in a random order.

2.3. Affymetrix platform-based microarray gene expression profiling

2.3.1. RNA processing

RNA isolation was performed using the Picopure™ RNA Isolation kit (Life Technologies, Grand Island, NY), with a DNase step (Qiagen, Valencia, CA). This typically resulted in approximately 1–25 ng of total RNA (Pietersen et al., 2009, 2011). The extracted RNA underwent two rounds of linear amplification using the RiboAmp® kit (Life Technologies, Grand Island, NY). A dilution of the resulting products (approximately 250 pg/μl) was used to determine the distribution of transcript lengths with the Experion StdSens Labchip (Bio-Rad Laboratories, Hercules, CA; Supplementary Fig. S1). The concentration and purity of these samples were determined by absorbance measurements at the optical density of A260 and A280, using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA).

2.3.2. Data analysis

Each array was scanned twice and the Affymetrix Microarray Suite 5.1 software averaged the two images to compute an intensity value for each probe cell within each probe set. For the quality control step, we employed the Partek® software's built-in function (Partek, St. Louis, MO). We then normalized the data with Partek's standard normalization method (i.e. data has a mean of zero and a variance of one, and each column for each sample was divided by the average of all control samples). Differentially expressed genes were visualized by performing unsupervised hierarchical clustering as stringency of the filtering criteria (i.e. fold-change and FDR-adjusted p-value) was varied to determine a representative gene list for pathway analyses.

Pathway analyses were performed with two web-based algorithms, *Ingenuity Pathway Analysis* (Ingenuity Systems, Redwood City, CA) and *MetaCore* (GeneGo, New York, NY), to map the differentially expressed genes onto biological functions and canonical pathways (Pietersen et al., 2014a,b). With *Ingenuity*, the significance for each of the identified pathways was determined via a Fisher's exact test, whereas GeneGo *Metacore* makes use of their algorithm for hypergeometric distribution, identifying pathways overrepresented with significant genes. Literature mining was then performed to elucidate the pathways or gene families that were particularly pertinent for oligodendrocyte functions.

2.3.3. Microarray and qRT-PCR validation

The TURBO Biotin labeling™ kit (end-labeling; Life Technologies, Grand Island, NY) was used to label the aRNA obtained from amplified samples. Gene expression profiling was performed using the Affymetrix

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