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Proteoglycan abnormalities in olfactory epithelium tissue from subjects diagnosed with schizophrenia

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ABSTRACT

Emerging evidence points to proteoglycan abnormalities in the pathophysiology of schizophrenia (SZ). In particular, markedly abnormal expression of chondroitin sulfate proteoglycans (CSPGs), key components of the extracellular matrix, was observed in the medial temporal lobe. CSPG functions, including regulation of neuronal differentiation and migration, are highly relevant to the pathophysiology of SZ. CSPGs may exert similar functions in the olfactory epithelium (OE), a continuously regenerating neural tissue that shows cell and molecular abnormalities in SZ. We tested the hypothesis that CSPG expression in OE may be altered in SZ. CSPG-positive cells in postmortem OE from non-psychiatric control ($n = 9$) and SZ ($n = 10$) subjects were counted using computer-assisted light microscopy. 'Cytoplasmic' CSPG (c-CSPG) labeling was detected in sustentacular cells and some olfactory receptor neurons (*c*-CSPG + ORNs), while 'pericellular' CSPG (p-CSPG) labeling was found in basal cells and some ORNs (*p*-CSPG + ORNs). Dual labeling for CSPG and markers for mature and immature ORNs suggests that *c*-CSPG + ORNs correspond to mature ORNs, and *p*-CSPG + ORNs to immature ORNs. Previous studies in the same cohort demonstrated that densities of mature ORNs were unaltered (Arnold et al., 2001). In the present study, numerical densities of c-CSPG + ORNs were significantly decreased in SZ ($p < 0.025$; 99.32% decrease), suggesting a reduction of CSPG expression in mature ORNs. Previous studies showed a striking increase in the ratios of immature neurons with respect to basal cells. In this study, we find that the ratio of p-CSPG + ORNs/CSPG + basal cells was significantly increased ($p = 0.03$) in SZ, while numerical density changes of p-CSPG + ORNs (110.71% increase) or CSPG + basal cells (53.71% decrease), did not reach statistical significance. Together, these results indicate that CSPG abnormalities are present in the OE of SZ and specifically point to a reduction of CSPG expression in mature ORNs in SZ. Given the role CSPGs play in OE cell differentiation and axon guidance, we suggest that altered CSPG expression may contribute to ORN lineage dysregulation, and olfactory identification abnormalities, observed in SZ.

1. Introduction

Chondroitin sulfate proteoglycans (CSPGs) play a key role in developmental and adult functions, such as axon guidance, cell adhesion, differentiation and migration, maturation of synapses and regulation of neurotransmitter receptor availability (Meyer-Puttlitz et al., 1996; Frischknecht et al., 2009; Maeda et al., 2010). These functions bear direct relevance to the pathophysiology of schizophrenia (SZ), a disease with a strong neurodevelopmental component (e.g. Arnold and Rioux, 2001; Harrison, 2007). Recently, significant CSPG expression anomalies have been detected in this disease (Buxbaum et al., 2008; Pantazopoulos et al., 2010; Enwright et al., 2012; Mauney et al., 2013). In particular, CSPG-enriched perineuronal nets were decreased in several brain regions, often in association with marked increased of CSPG-positive glial cells (Pantazopoulos et al., 2010; Enwright et al., 2012; Mauney et al., 2013). Together, these abnormalities have been postulated to disrupt neurodevelopmental processes, including neuronal migration, circuit formation and consolidation (Berretta, 2012).

Abbreviations: BSA, bovine albumin serum; CPZ, chlorpromazine-equivalent; c-CSPG, cytoplasmic chondroitin sulfate proteoglycan; CSPG, chondroitin sulfate proteoglycan; GAG, glycosaminoglycan; GAP43, growth associate protein 43; NSCP, neural stem/cell progenitor; OE, olfactory epithelium; OMP, olfactory marker protein; ORN, olfactory receptor neuron; PBS–Tx, phosphate buffer–Triton X; p-CSPG, pericellular chondroitin sulfate proteoglycan; PMI, postmortem time interval; RE, respiratory epithelium; RPTPz, receptor tyrosine phosphatase zeta; SCID, Structured Clinical Interview for DSM Disorders; SZ, schizophrenia; WFA, *Wisteria floribunda* agglutinin.

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CSPG developmental functions are thought to play a key role throughout life in the olfactory epithelium (OE), a neural structure in which neuronal differentiation, migration and axon outgrowth occur robustly throughout life (Clarris et al., 2000; Schwob, 2002). The adult OE contains stem cells that retain the capacity to divide and differentiate into mature olfactory receptor neurons (ORNs) (Schwob, 2002). During the course of their maturation, newly formed ORN axons join odor-specific axon bundles to reach the corresponding olfactory bulb glomeruli (Graziadei, 1973; Yoshihara and Mori, 1997; Beites et al., 2005). In the OE and olfactory bulb, development-specific patterns of CSPG expression help position ingrowing olfactory axons in the glomerular layer and maintain glomerular integrity (Gonzalez and Silver, 1994; Clarris et al., 2000). CSPG role in regulating brain cell differentiation (Yanagisawa and Yu, 2007; Purushothaman et al., 2012) suggests that they may contribute to OE basal cell differentiation and their maturation into ORNs.

OE abnormalities observed in SZ are consistent with CSPG dysregulation. Primary cell lines from OE biopsies were reported to have reduced adhesion properties and altered cell proliferation in SZ (Feron et al., 1999; McCurdy et al., 2006; Fan et al., 2012). Notably, disturbances of OE cell cycle include lower density of basal cells and increase of post-mitotic immature ORNs, providing strong support for a dysregulation of OE neuronal lineage (Feron et al., 1999; Arnold et al., 2001; Perry et al., 2002; McCurdy et al., 2006). Taken together, CSPG anomalies in several neural regions and abnormalities in the OE of SZs suggest a disruption of key CSPG functions in this structure. We tested the hypothesis that CSPG expression may be disrupted in the OE of subjects with SZ. A broad spectrum CSPG histological marker, i.e. Wisteria floribunda agglutinin (WFA) was used for group comparisons in postmortem OE tissue; antibodies raised against phosphacan and versican V0/V1, CSPGs suspected to be involved in SZ and to be expressed in the OE, were added for normal investigations on cell-specific CSPG distribution (Clarris et al., 2000; Popp et al., 2003; Buxbaum et al., 2008; Takahashi et al., 2011) (Pantazopoulos et al., unpublished observations).

2. Methods

2.1. Human subjects and tissue processing

2.1.1. Postmortem and biopsy human OE tissue for normal study

2.1.1.1. Postmortem. A tissue block containing the OE, cribriform plate, olfactory bulbs, lateral nasal walls and septum from a healthy control subject (male, 80 years old) was obtained from National Disease Research Interchange. The tissue block was processed as previously described (Holbrook et al., 2011). Sections were cut at 10 μm and mounted on super frost plus slides.

2.1.1.2. Biopsy. Tissue samples containing the OE from two healthy controls (males — 23 and 41 years of age) were obtained by biopsy under local anesthesia. Medical history, current medical status and absence of psychiatric disorders (Structured Clinical Interview for DSM Disorders, SCID) were recorded for each subject. Protocols for recruitment, consent, and biopsy were approved by the Institutional Review Boards of McLean Hospital and Massachusetts Eye and Ear Infirmary. Subjects provided written informed consent prior to their inclusion in the study. Biopsy samples were postfixed in 4% paraformaldehyde for 1 h, cryoprotected in 30% sucrose overnight and sectioned on a cryostat (10 μm).

2.1.2. Postmortem human OE tissue for group comparisons

Postmortem OE tissue was collected from 10 chronic SZ patients and 9 age- and sex-matched non-psychiatric controls (Table 1). All subjects with SZ were prospectively accrued from two state hospitals in Pennsylvania and were clinically assessed and diagnosed according to DSM-IV criteria by research psychiatrists of the University of Pennsylvania's Schizophrenia Mental Health Clinical Research Center, Philadelphia, as previously described (Arnold et al., 2001). This involved a standardized medical record review of demographic variables, presenting and subsequent symptoms, treatment history, medical history, caregivers' interview and laboratory and neuroimaging findings. Based on all information, diagnoses and inclusion were established by research team consensus. Non-psychiatric controls were obtained through the University of Pennsylvania's Alzheimer Disease Core Center. Review of clinical histories found no evidence of prior major psychiatric or neurological illnesses. Gross and microscopic diagnostic neuropathologic examinations of multiple cortical and subcortical regions revealed no evidence for changes consistent with Alzheimer's disease or cerebrovascular accidents in any of the subjects included in this cohort. At autopsy, the nasal epithelium, bony septae, and contiguous cribriform plate were removed *en bloc* and processed as above (Arnold et al., 2001).

2.2. Histochemistry and immunohistochemistry

2.2.1. Single immunohistochemistry/histochemistry for CSPGs

Tissue sections were blocked in 2% bovine albumin serum (BSA). For immunohistochemistry, sections were incubated for 48 h in

Table 1
Sample demographic and descriptive characteristics — comparison study. Data relative to subject cohort used for comparison studies. Brain weight expressed in grams. Abbreviations: CPZ, chlorpromazine-equivalent (CPZ) dose (expressed in average mg/day during the last month of life); Dx, diagnosis; PMI, postmortem interval (hours).

Dx	Age	Sex	PMI	Brain weight	CPZ last month of life	Age at onset	Years of illness
SZ	83	M	7	1200	0	21	62
SZ	74	F	15	1140	600	18	56
SZ	79	F	8	1040	0	NA	NA
SZ	75	F	16	1219	900	23	52
SZ	76	M	10	1322	600	20	56
SZ	70	M	17	1200	850	32	38
SZ	72	F	10	1157	NA	24	48
SZ	71	M	10	1188	0	34	37
SZ	74	M	14	1260	100	23	51
SZ	80	F	11	1060	100	22	58
10	**75.4 +/− 4.2**	**5 M/5 F**	**11.8 +/− 3.5**	**1178.6 +/− 85.0**	**350 +/− 382.4**	**24.11 +/− 5.4**	**50.89 +/− 8.6**
C	43	M	30.5	1545	NA	NA	NA
C	73	M	8	1249	NA	NA	NA
C	74	F	3.5	1000	NA	NA	NA
C	91	F	11.5	1140	NA	NA	NA
C	74	F	6	1250	NA	NA	NA
C	69	M	11	1625	NA	NA	NA
C	63	M	5	1360	NA	NA	NA
C	67	F	5.5	1100	NA	NA	NA
C	98	M	15	1340	NA	NA	NA
9	**72.4 +/− 15.8**	**5 M/4 F**	**10.7 +/− 8.3**	**1289.9 +/− 203.3**	**NA**	**NA**	**NA**