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Increased extracellular clusterin in the prefrontal cortex in schizophrenia

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ABSTRACT

The expression of the gene that encodes clusterin, a glycoprotein that has been implicated in the regulation of many cellular processes, has previously been found in gene expression profiling studies to be among the most significantly differentially expressed genes in pyramidal and parvalbumin-containing inhibitory neurons in the cerebral cortex in subjects with schizophrenia. In this study, we investigated whether clusterin may also be dysregulated at the protein level in schizophrenia subjects. We found that, although the intracellular amount of clusterin may be unchanged, the level of extracellular, secreted clusterin appears to be significantly increased in schizophrenia subjects. It is speculated that this finding may represent a neuroprotective response to pathophysiological events that underlie schizophrenia.

1. Introduction

Clusterin, also known as apolipoprotein J, is a rather ubiquitous multifunctional glycoprotein that has been shown to be involved in a multitude of cellular processes, such as maturation, differentiation, remodeling, transportation, proliferation, survival and death, in multiple organ systems, including the brain (Jones and Jomary, 2002; Klock et al., 2009; Rosenberg and Silkensen, 1995). In addition, clusterin dysregulation has been implicated in the pathogenesis of various human disorders, from tumorigenesis to neurodegenerative diseases, such as Alzheimer's disease, by acting to either promote or inhibit, among other events, oxidative stress, apoptosis, and synaptic plasticity (Charnay et al., 2012; Koltai, 2014; Park et al., 2014; Yu and Tan, 2012).

Clusterin exists in at least two sets of isoforms that exhibit distinct cellular and subcellular localizations (Rizzi et al., 2009; Yu and Tan, 2012). The secreted forms (~75–80 kDa) of clusterin, which are partially to heavily glycosylated, exhibit pro-survival functions and, additionally, have been shown to promote neuronal network complexity (Kang et al., 2005; Rizzi and Bettuzzi, 2010; White et al., 2001; Wicher et al., 2008; Yu and Tan, 2012). Conversely, the non-secreted, nuclear forms (~45–60 kDa) of unglycosylated clusterin appear to exert pro-apoptotic functions (Han et al., 2001; Kim and Choi, 2011; Kim et al., 2012; Rizzi and Bettuzzi, 2010).

Recent gene expression profiling studies suggest that clusterin is one of the most differentially regulated genes in schizophrenia. Specifically, we have found that clusterin gene expression appears to be upregulated by more than 2-fold in both pyramidal and parvalbumin-containing inhibitory neurons in the cerebral cortex in subjects with schizophrenia (Pietersen et al., 2014a, 2014b). In this study, we investigated whether clusterin protein expression might also be increased in this disorder. We found that although the density of cells that contained clusterin was not altered, the amount of the secreted, extracellular clusterin was in fact significantly increased in schizophrenia subjects. Given the pro-survival functions of the secreted clusterin, this increase may represent a homeostatic consequence of cellular injury mediated by, among other possible events, oxidative stress, which has recently been strongly implicated in the pathophysiology of schizophrenia (Bitanihirwe and Woo, 2011; Do et al., 2009, 2015; Fournier et al., 2014; Gysin et al., 2007; Monin et al., 2014).

2. Materials and methods

2.1. Postmortem human brain tissue

A total of 30 postmortem human brains were included in this study. Liquid nitrogen vapor fresh-frozen issue blocks containing Brodmann's area 9 of the prefrontal cortex from 15 schizophrenia and 15 normal control subjects, matched for age, sex and postmortem interval (PMI), were obtained from the Harvard Brain Tissue Resource Center (HBTRC) at McLean Hospital in Belmont, MA (Table 1). Postmortem human brain collection procedures at the HBTRC 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 histories. All of the brains included in this study were also examined by a

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Table 1
Cases included in this study.

Diagnosis	Age	Sex	PMI
Schizophrenia	60	M	22.17
Schizophrenia	77	M	25.33
Schizophrenia	48	F	29.92
Schizophrenia	32	M	38.43
Schizophrenia	56	F	26.55
Schizophrenia	58	M	25.33
Schizophrenia	47	F	31.85
Schizophrenia	65	M	21.06
Schizophrenia	59	M	29.67
Schizophrenia	69	F	23.08
Schizophrenia	58	F	25
Schizophrenia	63	M	26.16
Schizophrenia	66	M	21.75
Schizophrenia	60	F	17.13
Schizophrenia	64	F	18.5
	58.8 ± 10.5	M:F = 8:7	25.4 ± 5.5
Control	62	M	26.07
Control	76	M	23.92
Control	47	F	25.83
Control	46	M	28.83
Control	53	F	34.5
Control	55	M	23.92
Control	51	F	30.62
Control	65	M	20.92
Control	57	M	35.52
Control	65	F	27.17
Control	61	M	21
Control	60	F	21.67
Control	62	M	17.92
Control	58	F	25.7
Control	63	F	23.5
	58.7 ± 7.7	M:F = 8:7	25.8 ± 4.9

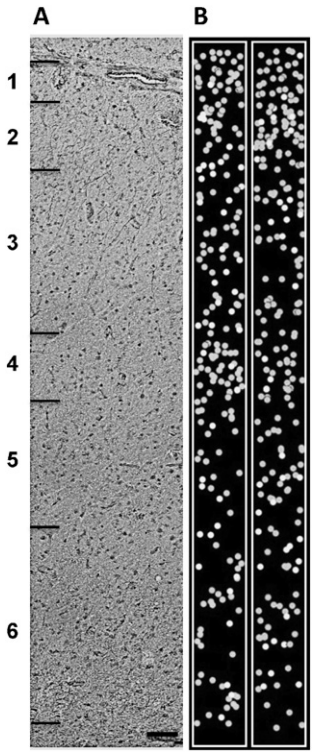

Fig. 1. A. Photomicrograph showing the distribution of clusterin-immunoreactive cells in the human prefrontal cortex in a control subject. Scale bar = 50 μm. **B**. Cell plots showing that the densities of cluster-immunoreactive cells are unaltered in schizophrenia (right) compared a normal control (left) subjects. Blue circles = non-pyramidal cells, yellow circles = glia, and green circles = pyramidal cells.

Board-certified neuropathologist to rule out any neurological conditions. In addition, the fact that none of the subjects had any history of active substance abuse or dependence disorder was confirmed by toxicological analysis.

2.2. Immunohistochemistry

Tissue blocks were sectioned at 20 μm, mounted on gelatin-subbed slides, and post-fixed in 4% paraformaldehyde for 20 min at room temperature. Sections were then incubated in endogenous enzyme block (1% H_2O_2, 10% MeOH) for 15 min and additionally blocked using 2% bovine serum albumin (BSA) with 10% normal goat serum (Life Technologies, 16210-064, Grand Island, NY) at room temperature for 1 h, followed by incubation in an anti-clusterin antibody produced in rabbit (1:100, SAB3500199, lot #38560504, Sigma-Aldrich, St. Louis, MO) at 4 °C overnight. Sections were then incubated at room temperature in biotinylated anti-rabbit IgG antibody produced in goat (1:500, BA-1000, Vector Labs, Burlingame, CA), followed by a 2-hour incubation in horseradish peroxidase-conjugated streptavidin (1:5000, Zymed, San Francisco, CA) made in 1 mol/L of phosphate buffer (PB) at room temperature, and finally in nickel-enhanced diaminobenzidine/peroxidase reaction (0.02% diaminobenzidine, 0.08% nickel-sulfate, 0.006% hydrogen peroxide in 1 mol/L PB). Sections were finally counterstained with cresyl violet, dehydrated and coverslipped.

Clusterin-immunoreactive elements comprised morphologically identifiable pyramidal, nonpyramidal, and glial cells. For each section, clusterin-immunoreactive cell within a 250 μm-wide cortical traverse containing all six cortical layers was quantified (Fig. 1). Quantification was performed in a blind fashion by one investigator (KA) using StereoInvestigator (MBF Bioscience, Williston, VT) and BioQuant (Nashville, TN) software.

2.3. Western blot

Tissue blocks consisting of only the gray matter were sectioned at 100 μm. Protein was isolated with radioimmunoprecipitation assay lysis buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% Na deoxycholate, 0.1% SDS, 10 mM EDTA, 100× Halt Protease Inhibitor Cocktail), sonicated 4 times at 30 Hz for 3 s with 15 s resting on ice, centrifuged at 13.2 RPM for 10 min at 4 °C, and frozen at −80 °C. A Bradford assay was completed in order to determine protein concentrations of each sample. Tissue samples were then run using NuPAGE 12% Bis-Tris Gel 1.0 mm × 10 well (NP0341BOX, lot #14072571, Carlsbad, CA) and transferred to immobilon-FL transfer membrane (IPFL00010, Lot #K4JA7438C, EMD Millipore, Billerica, MA), using powerease 500 supply (Invitrogen, Life Technologies, Singapore).

Blots were blocked using with LI-COR blocking buffer (927-4000, LI-COR Biosciences, Lincoln, NE) and 0.01 mol/L PBS with 0.05% Tween for 1 h at room temperature (1:1) before co-incubating overnight at 4 °C in an anti-clusterin antibody produced in rabbit (1:1000, SAB3500199, lot #38560504, Sigma-Aldrich, St. Louis, MO) and loading control anti-valosin-containing protein antibody made in mouse (VCP; 1:2500, ab11433, ABCAM, Cambridge, MA). Membranes were washed in 0.01 mol/L PBS with 0.05% Tween before co-incubating for 2 h at room temperature in the secondary goat anti-rabbit antibody (IRDye 800CW, 827-08365, lot #C40902-01, LI-COR Biosciences, Lincoln, NE) and donkey anti-mouse antibody (IRDye 680RD, 926-68072, lot #404910-05, LI-COR Biosciences, Lincoln, NE). Individual bands from all subjects were measured using Odyssey 3.0 analytical software (LI-COR Biosciences, Lincoln, NE) and normalized to VCP as a loading control. VCP has previously been shown to be unaltered in schizophrenia subjects (Bauer et al., 2009). Clusterin (secreted form) (SRP8004, Sigma-Aldrich, St. Louis, MO) was included as a standardized control on each blot to control for inter-blot variability. For quantification, the intensity of