Schizophrenia Research xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Abnormal expression of ER quality control and ER associated degradation proteins in the dorsolateral prefrontal cortex in schizophrenia

Pitna Kim *, Madeline R. Scott, James H. Meador-Woodruff

Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

ARTICLE INFO

Article history:
Received 6 July 2017
Received in revised form 27 November 2017
Accepted 12 February 2018
Available online xxxx

Keywords:
Postmortem brain
SYVN1/HRD1-SEL1L ubiquitin ligase complex
ERQC
ERAD

ABSTRACT

Abnormalities in posttranslational protein modifications (PTMs) that regulate protein targeting, trafficking, synthesis, and function have been implicated in the pathophysiology of schizophrenia. The endoplasmic reticulum (ER) contains specialized machinery that facilitate protein synthesis, ER entry and exit, quality control, and post-translational processing, steps required for protein maturation. Dysregulation of these systems could represent potential mechanisms for abnormalities of neurotransmitter associated proteins in schizophrenia. We hypothesized that expression of ER processing pathways is dysregulated in schizophrenia. We characterized protein and complex expression of essential components from protein folding, ER quality control (ERQC), and ER associated degradation (ERAD) processes in the dorsolateral prefrontal cortex of 12 matched pairs of elderly schizophrenia and comparison subjects. We found increased expression of proteins associated with recognizing and modifying misfolded proteins, including UDP-glucose/glycoprotein glucosyltransferase 2 (UGGT2), ER degradation enhancing alpha-mannosidase like protein 2 (EDEM2), and synoviolin (SYVN1)/HRD1. As SYVN1/HRD1 is a component of the ubiquitin ligase HRD1-SEL1L complex that facilitates ERAD, we immunoprecipitated SEL1L and measured expression of other proteins in this complex. In schizophrenia, SYVN1/HRD1 and OS-9, ERAD promoters, have increased association with SEL1L, while XTP3-B, which can prevent ERAD of substrates, has decreased association. Abnormal expression of proteins associated with ERQC and ERAD suggests dysregulation in ER localized protein processing pathways in schizophrenia. Interestingly, the deficits we found are not in the protein processing machinery itself, but in proteins that recognize and target incompletely or misfolded proteins. These changes may reflect potential mechanisms of abnormal neurotransmitter associated protein expression previously observed in schizophrenia.

1. Introduction

While the pathophysiology of schizophrenia is not well-understood, many hypotheses focus on abnormalities of neurotransmission in this illness. The dopamine, serotonin, glutamate, and GABA neurotransmitter systems have all been implicated in schizophrenia (Baou et al., 2016; Gonzalez-Burgos and Lewis, 2012; Howes and Kapur, 2009; Nakazawa et al., 2012), and the plurality of abnormalities of neurotransmitter associated proteins suggests dysfunction of cellular processes common to the regulation of these systems. Multiple abnormalities in expression of receptors and transporters associated with glutamate and GABA signaling, including abnormal N-glycosylation, receptor localization, and expression of endoplasmic reticulum (ER) - retention signals suggest dysfunctional processing of neurotransmitter associated proteins in the ER (Bauer et al., 2010; Kristiansen et al., 2010; Mueller et al., 2014; Mueller et al., 2015; Tucholski et al., 2013; Tucholski et al., 2013).

The ER is the first compartment in the intracellular secretory pathway, through which the majority of secreted and intramembrane proteins pass (Ellgaard and Helenius, 2003; Vembar and Brodsky, 2008). Protein synthesis, folding, and post-translational processing occur within the ER and are essential for proper protein maturation (Ellgaard and Helenius, 2003; Vembar and Brodsky, 2008). As the initial stage in protein synthesis and processing, the ER contains a highly regulated system for recognizing unfolded and misfolded proteins (Ellgaard and Helenius, 2003; Vembar and Brodsky, 2008). Folding of nascent polypeptide chains in the ER is monitored and regulated by chaperone proteins and folding enzymes, which facilitate the folding and assembly of newly synthesized proteins (Ellgaard and Helenius, 2003; Vembar and Brodsky, 2008). Many newly synthesized proteins are glycosylated, and transfer of N-glycans occurs in a single enzymatic step. The two outermost glucose residues of the N-glycans are rapidly and sequentially removed by mannosyl-oligosaccharide glucosidase (MOGS, also called glucosidase I) and glucosidase II (Araki and Nagata, 2012; Grinna and Robbins, 1979). Glucosidase II is composed of a catalytic subunit, neutral

* Corresponding author at: 1719 6th Avenue South, CIRC 590C, Birmingham, AL 35294-0021, USA.
E-mail address: pitnakim@uabmc.edu (P. Kim).

https://doi.org/10.1016/j.schres.2018.02.010
0920-9964/

alpha glucosidase AB (GANAB), and a regulatory subunit, glucosidase 2 subunit beta (PRKCSH) (Varki, 2009). The resulting N-glycan is recognized by the ER lectin-like chaperones calnexin (CNX) and/or calreticulin (CRT), which promote proper folding by protecting glycoproteins from aggregation or premature export from the ER(Araki and Nagata, 2012; Rutkevich and Williams, 2011; Williams, 2006). UDP-glucose/glycoprotein glucosyltransferase (UGGT) senses the folding state of glycoproteins released by CNX/CRT and targets correctly folded and assembled proteins for export to the Golgi (Ellgaard and Helenius, 2003; Vembar and Brodsky, 2008). If proteins are not correctly folded and assembled, UGGT reglucosylates the N-glycan to be reengaged by CNX/CRT (D'Alessio et al., 2010; Solda et al., 2007). Unfolded and terminally misfolded proteins within the CNX/CRT cycle are recognized, modified, and extracted from the ER for degradation by the ubiquitin (UB) proteasome system (UPS) (Ellgaard and Helenius, 2003), by the process of ER associated degradation (ERAD). ERAD glycoprotein substrates are recognized and modified by the lectins EDEM1/2/3, followed by association and modification by substrate recognition complexes including chaperones (GRP78, GRP94), lectins (OS.9, XTP3-B), and reductases (Christianson et al., 2011; Cormier et al., 2009; Maattanen et al., 2010; Ninagawa et al., 2014; Satoh et al., 2010; Vembar and Brodsky, 2008). OS-9 and XTP3-B directly bind with SEL1L, an adaptor protein that is essential for maintaining interaction with the E3 ubiquitin-protein ligase synoviolin (SYVN1; the human homolog of the E3 ligase HRD1 expressed in mice and yeast) (Christianson et al., 2011; Christianson et al., 2008). Substrates targeted to this complex are ubiquitinated by SYVN1/HRD1, a process that facilitates substrate recognition by the proteasome for degradation (MacGurn et al., 2012). The SYVN1/HRD1 UB ligase complex interacts with substrate extraction machinery (Christianson et al., 2008; St Pierre and Nabi, 2012; Vembar and Brodsky, 2008), including the transmembrane proteins derlin-1 and derlin-2 (DERL1/2), which eject substrates from the ER by forming a pore in the membrane (St Pierre and Nabi, 2012; Vembar and Brodsky, 2008). VCP-interacting membrane protein (VIMP) interacts with both DERLs and acts as a recruitment factor for cytosolic VCP-containing complexes (Christianson et al., 2011; Lee et al., 2015). VCP is an AAA-ATPase that is essential for substrate extraction from the ER into the cytosol in an ATP-dependent manner, where they are then degraded by the proteasome (Christianson et al., 2011; Zhong et al., 2004).

ER protein processing, ERQC, and ERAD are particularly important in neurons, and multiple reports suggest that Alzheimer's disease (AD) (Hoozemans et al., 2009; Hoozemans et al., 2005), Parkinson's disease (PD) (Hoozemans et al., 2007; Ryu et al., 2002), amyotrophic lateral sclerosis (ALS) (Atkin et al., 2008), Huntington's disease (HD) (Carnemolla et al., 2009; Duennwald and Lindquist, 2008), and Creutzfeldt Jacob disease (CJD) (Hetz et al., 2003) are all associated with ER stress and associated dysregulation. ER dysfunction has also been implicated in mood disorders and schizophrenia (Bown et al., 2000; Gold et al., 2013; Hunsberger et al., 2011; Nevell et al., 2014; So et al., 2007). To further characterize potential ER-associated abnormalities, we hypothesized that in schizophrenia there is dysregulation of ER protein processing pathways and proteins associated with protein folding, ERQC, and ERAD. This study sought to measure protein and complex expression of essential components of these processes in schizophrenia brain.

2. Materials and methods

2.1. Subjects

Samples were obtained from the Mount Sinai/Bronx Veterans Administration Medical Center brain collection. Assessment, consent, and postmortem procedures were conducted as required by the Institutional Review Boards of Pilgrim Psychiatric Center, Mount Sinai School of Medicine, and the Bronx Veterans Administration Medical Center. Patients were diagnosed with schizophrenia by two clinicians using DSM-III-R criteria, and had a documented history of psychiatric symptoms before the age of 40, as well as 10 or more years of hospitalization. Criteria for subject exclusion included a history of substance abuse, death by suicide, or coma for >6 h prior to death. Comparison subjects had no evidence of neuropathology, or signs of neurodegenerative disorders including Alzheimer's disease at assessment (Funk et al., 2012; Hammond et al., 2010; Mueller et al., 2014; Powchik et al., 1998; Purohit et al., 1998). Whole brains were dissected into 10 mm slabs in coronal plane. Grey matter from the dorsolateral prefrontal cortex (DLPFC, Brodmann areas 9/46) was blocked into 1 cm cubes from 12 patients with schizophrenia and matched comparison subjects (Table 1 and Supplementary Table S1) and stored at −80 °C until use.

2.2. Sample preparation

Tissue samples were reconstituted on ice in 5 mM Tris-HCl pH 7.5, 0.32 mM sucrose with a protease inhibitor tablet and a phosphatase inhibitor tablet (Complete Mini, EDTA-free and PhosSTOP, both from Roche Diagnostics, Mannheim, Germany) using a Power Gen 125 tissue homogenizer (Thermo Fisher Scientific, Rockford, Illinois). Protein concentration was determined using a BCA protein assay kit (Thermo Scientific, Rockford, Illinois). After homogenization, samples were stored at −80 °C until use.

2.3. SEL1L co-immunoprecipitation (Co-IP)

A 300 μg aliquot of each sample homogenate was used for a SEL1L Co-IP using M-280 sheep anti-mouse IgG Dynabeads (Life Technologies, Grand Island, NY). These beads are magnetic, permitting the use of a magnet to separate the supernatant from the beads after each incubation or washing step. To remove non-specific protein binding, a pre-clear step was performed by incubating the sample with 100 μl of beads for 1 h at 4 °C. 100 μl of fresh beads were first washed with cold Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST) and then blocked with TBST containing 0.1% BSA for 30 min at room temperature (RT) to decrease non-specific binding. Following this, the beads were incubated with rotation at RT for 1 h with 5 μg of anti-SEL1L antibody (LS Bio, # LS-B2253). Antibody-containing supernatant was then removed and excess antibody washed off with TBST. The antibody-bound beads were incubated rotaing for 1 h at RT with the pre-cleared sample homogenate from each subject. The supernatant was removed and the beads were washed with TBST to remove unbound protein. Finally, the beads were incubated in 2× loading buffer (0.2 M Tris-hydrochloride with 12% glycerol, 1.5% sodium dodecyl sulfate (SDS), and 0.7% BME) for 10 min at 70 °C to elute the bound protein off of the beads. The supernatant was then transferred to a new tube and stored at −20 °C until further use.

2.4. Western blot analysis

Target proteins of interest were quantified from schizophrenia and comparison subject pairs using western blot analysis. Samples were diluted in milli-Q water and 6× reducing buffer (4.5% SDS, 15% β-

Table 1
Summary of subject demographics.

	Comparison	Schizophrenia
n	12	12
Age	73.67 ± 9.04	75.83 ± 10.39
Sex	6 M/6F	6 M/6F
PMI (hours)	7.76 ± 7.54	13.58 ± 6.08
Tissue pH	6.43 ± 0.27	6.46 ± 0.20
On/off Rx	0/12	6/4

Abbreviations: Postmortem Interval (PMI); Cause of death (COD); Rx: On = treated with antipsychotic medications at time of death; Off = off antipsychotic medication for ≥6 weeks prior to death.

Please cite this article as: Kim, P., et al., Abnormal expression of ER quality control and ER associated degradation proteins in the dorsolateral prefrontal cortex in schizoph..., Schizophr. Res. (2018), https://doi.org/10.1016/j.schres.2018.02.010