Journal of Psychiatric Research 90 (2017) 31–39

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Alterations of ubiquitin related proteins in the pathology and development of schizophrenia: Evidence from human and animal studies

Jessica L. Andrews [a, b, c], Frederic J. Goodfellow [a, b], Natalie Matosin [a, b, c], Mollie K. Snelling [a, b], Kelly A. Newell [a, b, c], Xu-Feng Huang [a, b, c], Francesca Fernandez-Enright [a, b, c, d, *]

[a] Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW 2522, Australia
[b] Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia
[c] Schizophrenia Research Institute, Sydney, NSW 2010, Australia
[d] Faculty of Social Sciences, University of Wollongong, Wollongong, NSW 2522, Australia

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
22 December 2016
Accepted 17 January 2017

Keywords:
Schizophrenia
Neurodevelopmental animal model
Human brain cohort
E2 ubiquitin conjugating enzyme
E3 ligases

ABSTRACT

Gene expression analyses in post-mortem schizophrenia brains suggest that a number of ubiquitin proteasome system (UPS) genes are associated with schizophrenia; however the status of UPS proteins in the schizophrenia brain is largely unknown. Ubiquitin related proteins are inherently involved in memory, neuronal survival and morphology, which are processes implicated in neurodevelopmental disorders such as schizophrenia. We examined levels of five UPS proteins (Protein Inhibitor of Activated STAT2 [PIAS2], F-Box and Leucine rich repeat protein 21 [FBXL21], Mouse Double Minute 2 homolog [MDM2], Ubiquitin Carboxyl-Terminal Hydrolase-L1 [UCHL1] and Ubiquitin Conjugating Enzyme E2D1 [UBE2D1]) involved in these neuronal processes, within the dorsolateral prefrontal cortex of post-mortem schizophrenia subjects and matched controls (n = 30/group), in addition to across neuro-developmental time-points (juvenile, adolescent and adult stages of life), utilizing a well-established neurodevelopmental phencyclidine (PCP) animal model of schizophrenia. We observed significant reductions in PIAS2, FBXL21 and MDM2 in schizophrenia subjects compared to controls (p-values ranging from 0.002 to 0.004). In our developmental PCP model, MDM2 protein was significantly reduced in adult PCP-treated rats compared to controls ($p = 0.034$). Additionally, FBXL21 ($p = 0.022$) and UCHL1 ($p = 0.022$) were significantly decreased, whilst UBE2D1 was increased ($p = 0.022$), in juvenile phencyclidine-treated rats compared to controls. This is the first study reporting alterations of UPS proteins in post-mortem human schizophrenia subjects and in a neurodevelopmental model of schizophrenia. The findings from this study provide strong support for a role of these UPS proteins in the pathology and development of schizophrenia.

1. Introduction

Schizophrenia is a complex mental disorder, with intricate neurobiological alterations in a multitude of protein systems (Guillozet-Bongaarts et al., 2014; Rubio et al., 2013). Dysfunction in metabolic protein pathways, notably within five essential cellular metabolic pathways including the ubiquitin proteasome system (UPS), have been previously reported in the dorsolateral prefrontal

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; DLPFC, dorsolateral prefrontal cortex; FBXL21, F-Box and Leucine rich repeat protein 21; MDM2, Mouse Double Minute 2 homolog; NMDA, N-methyl-D-aspartate; PCP, phencyclidine; PFC, prefrontal cortex; PIAS2, Protein Inhibitor of Activated STAT2; PMI, post-mortem interval; PN, postnatal days; PSD-95, post-synaptic density protein-95; RIN, RNA integrity; UBE2D1, Ubiquitin Conjugating Enzyme E2D1; UCHL1, Ubiquitin Carboxyl-Terminal Hydrolase-L1; UPS, ubiquitin proteasome system.

* Corresponding author. Illawarra Health and Medical Research Institute, Building 32 Northfields Avenue, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia.

E-mail addresses: ja393@uowmail.edu.au (J.L. Andrews), fjg399@uowmail.edu.au (F.J. Goodfellow), natalie_matosin@uow.edu.au (N. Matosin), ms621@uowmail.edu.au (M.K. Snelling), knewell@uow.edu.au (K.A. Newell), xhuang@uow.edu.au (X.-F. Huang), fernande@uow.edu.au (F. Fernandez-Enright).

http://dx.doi.org/10.1016/j.jpsychires.2017.01.009
0022-3956/

cortex (DLPFC) from schizophrenia sufferers (Bousman et al., 2010; Rubio et al., 2013), and have also been correlated with synaptic changes, potentially implicated in schizophrenia-induced cognitive impairment (Glantz and Lewis, 2000; Goldman-Rakic, 1994; Petroski, 2008; Selemon and Goldman-Rakic, 1999). Gene expression profiling studies have consistently shown decreased expression in five cellular metabolic pathways in the prefrontal cortex (PFC) of schizophrenia patients, including the UPS pathways (Middleton et al., 2002). The DLPFC is a brain region highly involved in cognitive and executive functions.

The UPS represents a large number of essential cellular metabolism enzymes (Tai and Schuman, 2008), tagging target proteins for degradation via the protein-transport machinery process (Tai and Schuman, 2008). Ubiquitin and ubiquitin-like proteins (e.g. SUMO) are first activated by an ubiquitin-activating enzyme E1, before being transferred to the ubiquitin-conjugating enzyme E2. Upon reaching the final enzyme, the ubiquitin protein ligase E3, the target substrate is recognized and labeled with ubiquitin and/or ubiquitin-like proteins. This process is repeated until a short chain is formed, with three or more ubiquitin and/or ubiquitin-like molecules targeted to the proteasome for degradation. Defects in this process have been implicated in the etiology of a number of neurodegenerative diseases, metabolic disorders, cancer, developmental deficiencies, immunity pathologies (Sakamoto, 2002), and more recently, in schizophrenia (Rubio et al., 2013).

The relevance of the UPS in the pathology and development of schizophrenia remain largely unexplored. Alterations in the gene expression of *Ubiquitin Carboxyl-Terminal Hydrolase-L1* (*UCHL1*) and *Ubiquitin Conjugating Enzyme E2D1* (*UBE2D1*) (Novikova et al., 2006), and genetic association of *F-Box and Leucine rich repeat protein 21 (FBXL21)* (Chen et al., 2008) have been reported in schizophrenia. However, other important proteins in the UPS, such as Protein Inhibitor of Activated STAT2 (PIAS2) and Mouse Double Minute 2 homolog (MDM2, or [human] HDM2) have not been examined in schizophrenia-relevant paradigms. Despite having not been explored in the context of schizophrenia, the genes coding for the UPS proteins MDM2 and PIAS2, as well as UBE2D1 have been found to be dysregulated in the DLPFC in a postmortem cohort for autism (Chow et al., 2012). Dysfunction in any one of these UPS proteins in early neurodevelopment can have long-term effects leading to critical alterations in brain function (Hamilton and Zito, 2013; Romero-Granados et al., 2011), potentially contributing to the emergence of cognitive dysfunction in schizophrenia.

A number of developmental neuropsychiatric disorders often co-exist, and have a substantial overlap in their clinical presentation and neurobiology; these include schizophrenia and autism spectrum disorder (Morgan et al., 2008; Rapoport et al., 2009; Stahlberg et al., 2004), schizophrenia and intellectual disability (Morgan et al., 2008), and autism and ADHD (Rommelse et al., 2010; Simonoff et al., 2008). Since UPS proteins have previously been found to be dysregulated in the DLPFC of some of these neurodevelopmental disorders which co-exist with schizophrenia (Chow et al., 2012), it seemed appropriate to examine our UPS proteins in the DLPFC in schizophrenia.

For the first time, we have explored the levels of UCHL1, UBE2D1, PIAS2, FBXL21 and MDM2 proteins in the DLPFC of postmortem schizophrenia subjects. Of the known UPS proteins, these five were chosen specifically due to their involvement in pathways underlying brain development and neuronal survival, as well as cognitive function (Fig. 1) (Loriol et al., 2012; Plant et al., 2011). Our research group and others, have previously shown that perinatal administration of phencyclidine (PCP) to rats at postnatal days (PN) 7, 9 and 11 induces schizophrenia-like alterations in sensorimotor gating, locomotor activity, working memory and social interaction in rats (du Bois et al., 2008; Harich et al., 2007; Wang et al., 2003; Wiley et al., 2003), and causes biochemical alterations similar to those observed in the schizophrenia brain (Liu et al., 2011; Wang et al., 2000). Thus to explore whether these proteins may be involved in the development of schizophrenia, we further examined these proteins in this well-established neurodevelopmental rat model.

2. Methods

2.1. Human brain tissue samples

Tissue from 30 schizophrenia subjects (including 5 schizoaffective subjects), diagnosed using the Diagnostic and Statistical Manual of Mental Disorders IV, and 30 control subjects matched for age at death, post-mortem interval (PMI), brain pH and RNA integrity (RIN) (Table 1), were obtained from the New South Wales Brain Bank Network. All subjects with schizophrenia were prescribed antipsychotics at the time of death and a lifetime chlorpromazine equivalent was calculated for each patient. Additional details regarding characterization of this cohort have been previously described (Weickert et al., 2010) and summarized (Supplementary Table ST1). All studies were approved by, and conducted according to the guidelines of the Human Research Ethics Committees at the University of Wollongong (HE 99/222) and the University of New South Wales (HREC 07261).

2.2. Human brain tissue preparation and immunoblotting

Samples were obtained from the middle one-third (rostrocaudally) of the middle frontal gyrus from coronal slabs anterior to the genu of the corpus callosum (Weickert et al., 2010) (Brodmann Area 46). Tissue was homogenized and stored at −80 °C until required for immunoblotting, as previously detailed (Fernandez-Enright et al., 2014). The primary polyclonal antibodies used for this study were: anti-PIAS2 (ab4902 Abcam), anti-FBXL21 (ab57302 Abcam), anti-UBE2D1 (ab58245 Abcam), anti-UCHL1 (Ab5937 Chemicon) and anti-MDM2 (ab38618 Abcam). Detailed immunoblot methods can be found in Supplementary Materials.

2.3. Animals

Timed pregnant Sprague Dawley rats were obtained at gestation day 14 from the Animal Resource Centre (Perth, WA, Australia). Rats were housed at 22 °C in a 12:12 h light dark cycle with food and water ad libitum. Pups were sexed on PN7 and randomly assigned to PCP or saline groups. Pups were weaned at PN24-28, and were housed in pairs according to treatment. This study was approved by the Animal Ethics Committee at The University of Wollongong (AE13/01), and was conducted according to the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.4. Perinatal PCP treatment and tissue preparation for immunoblotting

Male rat pups (n = 6) were administered a subcutaneous injection of PCP (10 mg/kg/day; Sigma) or saline (0.9% NaCl) at a volume of 1 ml/kg on PN7, 9 and 11. This dosage and administration protocol was chosen due to PCP treatment inducing alterations in sensorimotor gating, locomotor activity and working memory (du Bois et al., 2008; Wang et al., 2003; Wiley et al., 2003). Additionally, the acute effects of PCP administration were validated by observing an immediate increase in locomotor activity and a lack of