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Decreased protein S-palmitoylation in dorsolateral prefrontal cortex in schizophrenia

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ABSTRACT

Recent reports suggest abnormalities of neurotransmitter receptor trafficking, targeting, dendritic localization, recycling, and degradation in the brain in schizophrenia. We hypothesized that a potential explanation for these findings may be abnormal posttranslational modifications that influence intracellular targeting and trafficking of proteins between subcellular compartments. Dysregulation of protein palmitoylation is a strong candidate for such a process. S-palmitoylation is a reversible thioesterification of palmitoyl-groups to cysteine residues that can regulate trafficking and targeting of intracellular proteins. Using a biotin switch assay to study S-palmitoylation of proteins in human postmortem brain, we identified a pattern of palmitoylated proteins that cluster into 17 bands of discrete molecular masses, including numerous proteins associated with receptor signal transduction. Using mass spectrometry, we identified 219 palmitoylated proteins in human frontal cortex, and individually validated palmitoylation status of a subset of these proteins. Next, we assayed protein palmitoylation in dorsolateral prefrontal cortex from 16 schizophrenia patients and paired comparison subjects. S-palmitoylation was significantly reduced for proteins in most of the 17 schizophrenia bands. In rats chronically treated with haloperidol, the same pattern of palmitoylation was observed but the extent of palmitoylation was unchanged, suggesting that the diminution in protein palmitoylation in schizophrenia is not due to chronic antipsychotic treatment. These results indicate there are changes in the extent of S-palmitoylation of many proteins in the frontal cortex in schizophrenia. Given the roles of this posttranslational modification, these data suggest a potential mechanism reconciling previous observations of abnormal intracellular targeting and trafficking of neurotransmitter receptors in this illness.

1. Introduction

Multiple hypotheses have implicated myriad neurotransmitter systems in the pathophysiology of schizophrenia. Recent findings suggest abnormal receptor localization may play a role in abnormalities of both excitatory and inhibitory neurotransmitter pathways in this illness. For example, alterations have been found in transcripts associated with AMPA receptor localization and regulation (Beneyto and Meador-Woodruff, 2006; Drummond et al., 2013), and expression of some glutamate receptor subunits within specific subcellular compartments have been shown to be abnormal in this illness (Hammond et al., 2010; Kristiansen et al., 2010). Additionally, extent of N-linked glycosylation is associated with subcellular localization; abnormal glycan expression associated with N-glycosylation of glutamatergic receptor subunits (Tucholski et al., 2013a, 2013b) excitatory amino acid transporters (Bauer et al., 2010), and $GABA_A$ receptor subunits (Mueller et al., 2014), suggests that abnormal subcellular distribution of such proteins may contribute to disturbances of neurotransmission in schizophrenia. Given that these abnormalities in subcellular localization are found in multiple neurotransmitter systems, we hypothesized that posttranslational protein modifications (PTM) affecting targeting and localization of proteins may be altered in schizophrenia.

The intracellular distribution of neurotransmitter receptors and transporters, as well as other proteins, can be regulated by the uniquely reversible PTM, S-palmitoylation (El-Husseini and Bredt, 2002; Fukata and Fukata, 2010). This is in large measure due to the membrane stability facilitated by the hydrophobic 16-carbon palmitic fatty acid that can be covalently bound to cysteine residues. Palmitoylation status is tightly orchestrated by a large family of enzymes responsible for the addition or removal of palmitic acid, resulting in a cycle of palmitoylation/depalmitoylation that provides a mechanism for multiple cellular

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processes including subcellular targeting of proteins (Aicart-Ramos et al., 2011). The palmitoyl moiety is initially bound by a thioester linkage to cysteine residues by members of a family of palmitoyl acyl transferases (PATs) (Mitchell et al., 2006). Palmitoyl groups are dynamically removed by depalmitoylating enzymes including acyl-protein thioesterases (APTs) and palmitoyl protein thioesterases (PPTs) (Conibear and Davis, 2010). This reversibility of palmitoylation provides cyclical membrane association functionally required for a diverse number of proteins.

Many proteins associated with neurotransmission are known to be palmitoylated, including some glutamatergic receptor subunits (Hayashi et al., 2005, 2009), GABA receptor subunits (Fang et al., 2006; Keller et al., 2004; Rathenberg et al., 2004), proteins involved in release and recycling of neurotransmitters (El-Husseini and Bredt, 2002), and proteins associated with regulating trafficking to and stability of receptors at the synaptic membrane (Aicart-Ramos et al., 2011; Dejanovic et al., 2014; El-Husseini et al., 2000a; Huang and El-Husseini, 2005; Linder and Deschenes, 2007; Smotrys and Linder, 2004).

Interestingly, DiGeorge syndrome, associated with genetic deletion at 22q11.2, is associated with a 20–30 fold increase in risk of developing schizophrenia (Drew et al., 2011; Mukai et al., 2004). A mouse model for human 22q11 syndrome (Mukai et al., 2008, 2015) has been shown to exhibit palmitoylation abnormalities. One of the deleted genes at 22q11.2 is a key palmitoyltransferase, ZDHHC8, which regulates proteins involved with axonal growth and arborization. ZDHHC8-deficient mice have impaired palmitoylation and axonal branching, which can be rescued by the reintroduction of active ZDHHC8 (Mukai et al., 2015). Palmitoylation has also been implicated in several neurodegenerative disorders (Antinone et al., 2013; Butland et al., 2014; Mitchell et al., 2014), but has yet to be well-characterized in human brain or studied in schizophrenia.

Given the role of palmitoylation in subcellular localization, and growing evidence for abnormalities in trafficking and targeting of proteins that regulate neurotransmission in schizophrenia, we hypothesized that abnormal protein palmitoylation could be an underlying mechanism contributing to the pathophysiology of schizophrenia. To address this, we first developed an assay to characterize protein palmitoylation in postmortem human brain, based on a previously described acyl-biotinyl exchange (ABE) technique (Drisdel and Green, 2004; Kang et al., 2008; Wan et al., 2007). Next, we studied extent of total protein palmitoylation in dorsolateral prefrontal cortex (DLPFC) in subjects with schizophrenia and paired comparison subjects. Given the integral roles palmitoylation plays in membrane targeting and interaction of proteins, we suggest that compromised protein palmitoylation may be a novel mechanism contributing to the abnormal subcellular targeting of proteins seen across multiple neurotransmitter pathways in schizophrenia brain.

2. Materials and methods

2.1. Human subjects

Samples of human brain used in this study were obtained from the Mount Sinai Medical Center brain collection (Table 1) as previously described (Davidson and Keefe, 1995; Funk et al., 2009; Harvey et al., 1992; Powchik et al., 1998). Schizophrenia patients had psychotic symptoms before the age of 40 and were hospitalized for at least 10 years. Patients were recruited prospectively and underwent extensive antemortem evaluation. Consent was obtained from the next

Table 1
Paired subject demographics.

Pair	Subject	Sex	Age	pH	PMI	R_x	Cause of death
1	Comparison	F	73	6.3	3.4		Ac. myocardial infarction
	Schizophrenia	F	74	6.3	7	On	Cardiopulmonary arrest
2	Comparison	F	74	6.0	3		Cardio respiratory failure
	Schizophrenia	F	76	6.1	8.5	On	Cardiopulmonary arrest, cancer of breast
3	Comparison	F	83	6.8	6.2		Cardiopulmonary arrest
	Schizophrenia	F	79	6.8	9.9	Off	Cardiac arrest
4	Comparison	F	92	6.2	3.5		
	Schizophrenia	F	89	6.2	9.6	On	Cardiopulmonary arrest
5	Comparison	F	82	6.1	5.7		Cardiopulmonary arrest
	Schizophrenia	F	81	5.9	12.5	Off	
6	Comparison	F	74	6.3	4.8		Cardiopulmonary arrest
	Schizophrenia	F	76	6.1	21.2	On	Cardiogenic shock
7	Comparison	F	75	6.0	6.5		
	Schizophrenia	F	76	6.0	9.7	On	Cardiopulmonary arrest
8	Comparison	M	58	6.7	12.3		Arteriosclerotic heart disease
	Schizophrenia	M	58	6.9	13.3	On	Cardiopulmonary failure
9	Comparison	M	59	6.7	20.4		Cardiopulmonary arrest
	Schizophrenia	M	56	6.5	13.5	Off	
10	Comparison	M	65	6.8	3.8		Renal failure
	Schizophrenia	M	68	6.8	5.6	On	Cardio respiratory failure
11	Comparison	M	64	6.3	4.2		
	Schizophrenia	M	63	6.3	6.2	On	Cardiopulmonary arrest
12	Comparison	M	75	6.3	16		
	Schizophrenia	M	73	6.5	7.9	On	Cardio respiratory failure
13	Comparison	M	92	6.4	20		
	Schizophrenia	M	93	6.6	17.7	Off	Cardiopulmonary arrest
14	Comparison	M	60	6.6	28.7		
	Schizophrenia	M	57	6.4	20.7	On	Acute myocardial infarction, ASHD
15	Comparison	M	76	6.3	2.9		Cardiopulmonary arrest
	Schizophrenia	M	77	6.4	24	Off	
16	Comparison	M	73	6.2	14.9		Cardio respiratory failure
	Schizophrenia	M	73	6.2	8.8	On	Cardiopulmonary arrest
	Comparison	Mean:	73 ± 10.5	6.4 ± 0.3	9.8 ± 8.0		
	Schizophrenia		73 ± 10.6	6.4 ± 0.3	12.3 ± 5.7		

Abbreviations: female (F); male (M); postmortem interval (PMI, hours); mean (group mean with SD); and on/off Rx: received antipsychotic medications within 6 weeks of death.

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