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Cell migration in schizophrenia: Patient-derived cells do not regulate motility in response to extracellular matrix

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ABSTRACT

Schizophrenia is a highly heritable psychiatric disorder linked to a large number of risk genes. The function of these genes in disease etiology is not fully understood but pathway analyses of genomic data suggest developmental dysregulation of cellular processes such as neuronal migration and axon guidance. Previous studies of patient-derived olfactory cells show them to be more motile than control-derived cells when grown on a fibronectin substrate, motility that is dependent on focal adhesion kinase signaling. The aim of this study was to investigate whether schizophrenia patient-derived cells are responsive to other extracellular matrix (ECM) proteins that bind integrin receptors. Olfactory neurosphere-derived cells from nine patients and nine matched controls were grown on ECM protein substrates at increasing concentrations and their movement was tracked for 24 h using automated high-throughput imaging. Control-derived cells increased their motility as the ECM substrate concentration increased, whereas patient-derived cell motility was little affected by ECM proteins. Patient and control cells had appropriate integrin receptors for these ECM substrates and detected them as shown by increases in focal adhesion number and size in response to ECM proteins, which also induced changes in cell morphology and cytoskeleton. These observations indicate that patient cells failed to translate the detection of ECM proteins into appropriate changes in cell motility. In a sense, patient cells act like a moving car whose accelerator is jammed, moving at the same speed without regard to the external environment. This focuses attention on cell motility regulation rather than speed as key to impairment of neuronal migration in the developing brain in schizophrenia.

1. Introduction

Neuropathological studies show widespread structural and cytoarchitectural differences in post-mortem brain in schizophrenia compared to healthy controls. The findings suggest that fundamental processes of brain development are involved and continue with age, with all cell types included: neurons, astrocytes and oligodendrocytes (Wright et al., 2000). One potentially affected process is cell migration, with several key regulators of neuronal migration implicated in schizophrenia: *DISC1* (Meyer and Morris, 2009; Steinecke et al., 2012; Tomita et al., 2011), *NRG1* (Sei et al., 2007) and *RELN* (Britto et al., 2014; D'Arcangelo et al., 1995). Cells migrate through the surrounding microenvironment of the extracellular matrix (ECM) and there is evidence that ECM proteins are altered in post-mortem brain in schizophrenia. For example, perineuronal net density was reduced in amygdala (Pantazopoulos et al., 2010), hippocampus (Shah and Lodge, 2013) and prefrontal cortex (Enwright et al., 2016); *post-mortem* patient brain showed increased numbers of chondroitin sulfate proteoglycan-expressing astrocytes (Pantazopoulos et al., 2010) and decreased numbers of aggrecan-labelled glia (Pantazopoulos et al., 2015); reelin protein expression was reduced in prefrontal cortex (Habl et al., 2012), hippocampus (Fatemi et al., 2000) and temporal cortex (Impagnatiello et al., 1998); and laminin and collagen levels are also reduced in *post-mortem* superior temporal cortex from schizophrenia patients (Schmitt et al., 2012). Additionally, chondroitin sulfate proteoglycan expression is reduced in *post-mortem* patient olfactory epithelium (Pantazopoulos et al., 2013). These findings open the possibility that *post mortem* cytoarchitectural differences in schizophrenia may be due in part to dysfunctional ECM-dependent neuronal migration.

Dysfunctional neuronal migration is also evident the analysis of 108 schizophrenia risk genes identified in a recent genome-wide association (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Clusters of these risk genes collectively converge into cellular pathways that control neuronal migration and cell adhesion (Aberg et al., 2013; Lips et al., 2012; O'Dushlaine et al., 2011). Defective adhesion is directly interlinked with cell migration and axon guidance, pathways that are also found to be abnormal among the schizophrenia risk genes (Gilman et al., 2012; Kahler et al., 2008; Perkins et al., 2007; Potkin et al., 2009). There is also a disease-dependent correlation in pathways for cytoskeletal actin dynamics

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and remodeling (Fromer et al., 2014; Zhao et al., 2014), key cytoskeletal proteins (Schmitt et al., 2012) and cell communication, which includes cell-cell and cell-ECM interactions (Cristino et al., 2014).

Patient-derived cells provide an opportunity to investigate directly such highly regulated cellular processes as neuronal migration. Several studies of schizophrenia patient-derived cells and tissues have now shown impaired cell adhesion and motility and other cellular processes that modulate cell migration. For example, patient-derived olfactory epithelium was less adherent than control-derived epithelium when plated onto fibronectin-coated plastic (Feron et al., 1999), a difference that was reduced when plated onto clean glass (McCurdy et al., 2006). Dysregulation of multiple signaling pathways associated with neuronal adhesion and migration was evident in gene expression of olfactory neurosphere-derived cells from schizophrenia patients compared to cells from healthy controls (Matigian et al., 2010). These same patient-derived cells moved faster than control-derived cells and had faster dynamics of focal adhesion turnover when patient cells were plated on fibronectin-coated plastic (Fan et al., 2013) but moved slower than control-derived cells on reelin (Tee et al., 2016). Neuronal precursors from primary cultures of olfactory epithelium also moved slower than control-derived cells (Munoz-Estrada et al., 2015). Similarly, neuronal precursors differentiated from patient-derived induced pluripotent stem cells moved slower than controls (Brennand et al., 2015; Topol et al., 2015). Our working hypothesis is that these differences in schizophrenia-associated motility may result from differences in the ECM protein substrates used, because the same olfactory neurosphere-derived stem cells from patients were more motile than controls when plated on fibronectin (Fan et al., 2013) but less motile when on reelin (Tee et al., 2016).

The aim of the present study was to investigate more fully the relationship between cell motility and ECM in olfactory neurosphere-derived cells from patients with schizophrenia and from healthy controls. Our hypothesis was that patient and control cells would respond differently to ECM protein concentrations leading to differences in cell motility. We quantified cell motility using automated imaging and analysis of living cells moving in multi-well plates coated with increasing concentrations of a variety of ECM proteins that stimulate a wide range of integrin receptors. Automated imaging and analysis methods were also used to investigate cell size and shape, and cellular components involved in cell adhesion and motility (*i.e.* integrin receptors, focal adhesions and cytoskeletal proteins). Use of multi-well plates allowed close control of many confounding variables within each experiment, hence reducing variability and providing large numbers of single cell replicates representing each of the nine patient-derived and nine control-derived cell lines used in this study.

2. Materials and methods

2.1. Healthy control and patient-derived cell lines

Patient-derived olfactory cell lines used in this study are the same as those used in our previous reports (English et al., 2015; Fan et al., 2012; Fan et al., 2013; Mar et al., 2011; Matigian et al., 2010; Tee et al., 2016). One of the control cell line (cell line ID: 100030002), was replaced with another cell line (ID: 10008017, Table 1.). Cell lines were generated from olfactory mucosa biopsies extracted from age-matched male donors who comprise schizophrenia patients ($N = 9$) and healthy controls ($N = 9$). Disease status of the patient cohort was classified based on the Diagnostic Interview for Psychosis (DIP), according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). All biopsies were approved by the Ethics Committee for West Moreton Region, Queensland Health and the Griffith University Human Ethics Committee (Queensland, Australia). As part of the approval process, all participants gave written, informed consent for their cells to be grown *in vitro*, banked and used for experiments to understand the biological bases of schizophrenia. The approved biopsy procedure and subsequent experiments were conducted according to the guidelines of the National Health and Medical Research Council of Australia. Participant details are presented in Table 1.

Table 1
Participant details.
Table adapted from Fan et al. (2013) and Tee et al. (2016). Data originally published in Matigian et al. (2010).

Cell line ID	Age	Sex	Medication	CPE equivalent	Cigarettes per day
Controls					
10008017	49	Male			
10002001	31	Male			
10002002	47	Male			10
10002003	28	Male			
10003001	17	Male			
10003003	32	Male			
10003004	46	Male			
10003005	56	Male			
10003006	45	Male			5
Patients					
30002001	46	Male	Clozapine: 250 mg/day Omeprazole magnesium: 20 mg/day	333	25
30002002	58	Male	Olanzapine: 7.5 mg/day Benztropine: 1 mg/day Diclofenac sodium: 100 mg/day	250	
30002003	21	Male	Quetiapine: 800 mg/day Paroxetine: 40 mg/day	1194	15
30002004	33	Male	Risperidone: 4 mg/day	267	
30002005	49	Male	Clozapine: 350 mg/day	467	
30002006	27	Male	Olanzapine: 16 mg/day	533	30
30002007	44	Male	Clozapine: 475 mg/day Lithium carbonate: 1250 mg/day Atenolol: 75 mg/day Aspirin: dose unknown	633	20
30002008	28	Male	Flupenthixol decanoate: 200 mg/month	500	10
30002009	38	Male	Risperidone: dose unknown	Unknown	60

CPE equivalent units calculated based on method published in Davis (1976).

2.2. Olfactory neurosphere-derived cell culture

In this study, the term “patient cells” describes cell lines generated from schizophrenia patients and “control cells” represent cell lines generated from healthy controls. The cells are “olfactory neurosphere-derived cells”, defined by their mode of generation (Feron et al., 2013). Primary cultures of olfactory mucosa were cultured in growth medium (Dulbecco's Modified Minimum Essential Medium, DMEM/F12; Gibco, Life Technologies, Grand Island, NY, USA; supplemented with 1% penicillin-streptomycin, Gibco, Life Technologies; and 10% fetal bovine serum, Bovogen, Keilor East, VIC). To induce the formation of neurospheres when the cultures were confluent, the medium was changed to serum free with added basic fibroblast growth factor (25 ng/ml) and epidermal growth factor (50 ng/ml). Free floating neurospheres were harvested every two days and subsequently grown in growth medium at 37 °C and 5% CO_2. All cell lines were previously cryopreserved as small aliquots and subsequently thawed and grown in the same growth medium. All experiments were conducted using cells between passages 5 to 10. All cells were grown on tissue culture plastic unless stated otherwise.

2.3. Cell cycle synchronization

At 80% confluence, growth medium was replaced with synchronization medium - DMEM/F12 supplemented with 0.5% FBS and 1% penicillin-streptomycin. Cells were cultured in synchronization medium for 48 h to keep all cells in the population at G1 cell cycle phase as previously described (Fan et al., 2012).