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Full-length Article

Syncytin-1, an endogenous retroviral protein, triggers the activation of CRP *via* TLR3 signal cascade in glial cells

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ABSTRACT

Schizophrenia is a devastating psychiatric disorder that impacts on social functioning and quality of life, and there is accumulating evidence that inflammation is a potential pathogenic mechanism of schizophrenia. However, the mechanism of inflammation possibly occurred in schizophrenia has not been well understood. The endogenous retroviral protein syncytin-1 and inflammatory marker CRP are both abnormally expressed in schizophrenia patients. CRP is one of the markers of bacterial infection generally. Less clear is whether virus or viral protein can trigger the activation of CRP. Here, we detected a robust increase of the levels of syncytin-1 and CRP in schizophrenia patients, and displayed a positive correlation and marked consistency between expressions of syncytin-1 and CRP in schizophrenia patients. Furthermore, overexpression of syncytin-1 significantly elevated the levels of CRP, TLR3, and IL-6 in both human microglia and astrocytes. TLR3 deficiency impaired the expressions of CRP and IL-6 induced by syncytin-1. Importantly, we observed a cellular co-localization and a direct interaction between syncytin-1 and TLR3. Additionally, knockdown of IL-6 inhibited the syncytin-1-induced CRP expression. Thus, the totality of these results showed that viral protein syncytin-1 could trigger the activation of CRP, which might explain the elevated CRP in sterile inflammation and exhibit a novel mechanism for regulation of inflammation by syncytin-1 in schizophrenia.

1. Introduction

Schizophrenia is one of the most devastating and prevalent psychiatric disorders, with the worldwide prevalence of approximately 1% (Perron et al., 2008). Characterized by abnormal brain functions including cognition, emotion, and perception (Lewis and Lieberman, 2000), schizophrenia brings a huge challenge to human health. Although the pathogenesis of schizophrenia is still not clear at present, an increasing number of studies had focused attention on the inflammation change in schizophrenia (Leza et al., 2015). Recent researches had demonstrated that abnormal expressions of inflammatory cytokines were involved in schizophrenia. For instance, interleukin-6 (IL-6), one of pro-inflammatory cytokines, was detected to be increased in the serum of schizophrenia patients (Potvin et al., 2008). C-reactive protein (CRP), an acute inflammatory marker of hepatic origin, was also found to be abnormally expressed in the serum of schizophrenia patients (Misiak et al., 2017). Meanwhile, serum IL-8 (Zhang and Chen, 2002) and MCP-1 (Mundo et al., 2005) which were associated with some autoimmune diseases, were significantly higher in schizophrenia patients than in normal controls. Additional evidence showed that the expressions of several Toll like receptors (TLRs) were substantially higher in the schizophrenia patients (Muller et al., 2012). TLRs are a type of pattern recognition receptors (PRRs), and recognize pathogen-associated molecular patterns

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(PAMPs) broadly shared by pathogens, which play a key role in the innate immune system. Activation of TLRs can lead to the production of inflammatory cytokines, inducing the generation of inflammation (Kawai and Akira, 2010). In spite of these observations, the exact mechanisms of regulating inflammation in microglia in schizophrenia still need to be further explored.

Increasing studies also indicated that human endogenous retrovirus W family (HERV-W), one family member of HERVs, was associated with schizophrenia (Christensen, 2010). HERV-W ENV which was also called ERVWE1 or syncytin-1, and encoded by the HERV-W envelope glycoprotein at chromosome 7, was reported in the literature to be significantly increased in schizophrenia patients (Huang et al., 2011). Syncytin-1, well known as an immunotoxin which can induce superantigen-like effects with potent inflammation, can lead to the activation of innate immune system (Perron et al., 2001). However, the specific mechanism that syncytin-1 regulates inflammation reaction remains unknown.

Here, our researches identified a positive correlation and marked consistency between the viral protein syncytin-1 and CRP in schizophrenia patients, and gave proofs that syncytin-1 could trigger the activation of CRP in glial cells. Importantly, we showed that syncytin-1 could directly interact with TLR3 to elevate the expression of TLR3, and trigger the activation of CRP through TLR3-IL-6 signaling pathway. Thus, on this basis we proposed a novel view that it might be HERV proteins such as syncytin-1 that induced the increase of CRP, which might explain the high expression of CRP in sterile inflammation in some neurologic diseases. Meanwhile, we provided potential clue to discover the molecular mechanism of inflammation possibly occurred in schizophrenia.

2. Methods

2.1. Blood samples

All patients and controls were enrolled in RenMin Hospital, Wuhan University. Blood samples (both sera and plasma samples) were collected from 99 patients and 83 normal individuals. The inclusion criteria of schizophrenia patients were the following: (1) The patients were randomly selected from the outpatients and inpatients of RenMin Hospital. (2) The patients exhibited symptoms consistent with recent-onset schizophrenia defined by the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Ed (DSM-IV). (3) The patients had not been admitted previously to any hospital for schizophrenia. Manifestations of acute infectious, inflammatory, and neurological diseases were the exclusion criteria. The median age of these individuals was 25 years old (ranging from 16 to 45). For controls, they were all healthy blood donors obtained from the health check-up in RenMin Hospital, Wuhan University. The 83 normal controls displayed no any evidence of neurological or psychiatric diseases, with age range of 20–43 years and a median age of 25 years. There were no significant differences in median age, ethnicity, geographic place of birth, body mass index and gender between control and schizophrenia patients. Blood sample collections were under consensus agreements, and were approved by the Institutional Review Board of Wuhan University, School of Medicine. All plasma samples were mixed with ethylenediaminetetraacetic acid. Samples were all stored at −80 °C immediately until experiments.

2.2. Nested polymerase chain reaction amplification

The blood samples of 83 normal individuals and 99 schizophrenia patients were used to amplify the mRNA of syncytin-1 by nest-PCR as described previously (Huang et al., 2011). Briefly, in the first round of amplification, the primers (Forward primer: 5′-GAAG TAATCTCCCAACTCA-3′; reverse primer: 5′-TTTCAGCGGTTAG CAAGT-3′) were used for polymerase chain reaction (PCR). Then one microliter of the PCR product was subjected to the second round PCR with primers (Forward primer: 5′-GCCTATTTAATAC CACCCT-3′; reverse primer: 5′-GAGCCATTCAAACAACGA-3′).

2.3. Plasmids and plasmids constructs

The syncytin-1 plasmid was obtained as previously described (Huang et al., 2011). Syncytin-1-TM, a transmembrane region sequence of syncytin-1 gene, was cloned into the pEGFP-C1 (the plasmid was termed pEGFP-syncytin-1-TM) to study the localization of syncytin-1. shRNA targeting the human *TLR3* (5′-G CAGTGTTGAACCTTACCCA-3′) or *IL-6* (5′-GGAGACATGTAACAA GAGT-3′) sequence was cloned into the psilencer 2.1-U6 neo plasmid. "Scrambled" shRNA (5′-GCGCUAGACUUAACGUAAUGA-3′) was used to control the non-specific effects of the transfected shRNA (the plasmid was termed shScram). The pcDNA3-TLR3-CFP plasmid was a gift from Dr. Douglas Golenbock (University of Massachusetts Medical School, Medicine, USA). For the luciferase assay, sequences containing the promoter region of human *TLR3* or *CRP* were cloned into the luciferase reporter vector pGL3-basic which contains a firefly luciferase gene, to produce pGL3-TLR3 and pGL3-CRP.

2.4. Cell culture and transfection

Several cell lines were used in this study, including human U251, CHME-5, A172, and HEK293T cell lines. Among them, U251 was the human astrocyte glioma cell line. Relatively, CHME-5 was the human microglia glioma cell line. A172 was human glioblastoma cell line. HEK293T was human embryonic kidney cells line. All cell lines were purchased from the American Type Culture Collection and maintained in DMEM (GIBCO, California, USA) supplemented with 10% FBS (GIBCO, California, USA) and 100 U/ml penicillin/streptomycin at 37 °C with 5% CO_2. Rat primary glial cells were obtained from the cerebral cortex of rat according to previously published methods (Fex Svenningsen et al., 2011; Wicher and Norlin, 2015). An immunofluorescence assay using microglial marker Iba-1 antibody was performed to identify the type of primary glial cells (Supplementary Fig. S1). Cells were transfected (at 80% confluence) using Lipofectamine® LTX and PLUS™ (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and were collected 48 h after transfection for further study.

2.5. Luciferase assay

HEK293T cells or U251 cells were co-transfected with pGL3-TLR3 promoter or pGL3-CRP promoter plasmid, pCMV-syncytin-1 or an empty plasmid pCMV, and a pRL-CMV Renilla luciferase vector. 48 h after transfection, the luciferase activities were measured using a Promega Dual Luciferase Reporter Assay (Promega, Madison, WI, USA) according to the manufacturer's instructions.

2.6. ELISA

Culture supernatants and intracellular fluid were harvested and preserved at −80 °C before evaluation of cytokine production by ELISA. OptEIA ELISA kits (Becton, Dickinson and Company, New Jersey, USA) of human IL-6, IL-8, MCP-1 and CRP were used. Experiments were carried out according to the kit manufacturers' instructions.

The ELISA assay for syncytin-1 was performed according the standard protocol (Perron et al., 2008). Briefly, we first coated

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