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Short communication

SFPQ associates to LSD1 and regulates the migration of newborn pyramidal neurons in the developing cerebral cortex

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ABSTRACT

The development of the cerebral cortex requires the coordination of multiple processes ranging from the proliferation of progenitors to the migration and establishment of connectivity of the newborn neurons. Epigenetic regulation carried out by the COREST/LSD1 complex has been identified as a mechanism that regulates the development of pyramidal neurons of the cerebral cortex. We now identify the association of the multifunctional RNA-binding protein SFPQ to LSD1 during the development of the cerebral cortex. *In vivo* reduction of SFPQ dosage by *in utero* electroporation of a shRNA results in impaired radial migration of newborn pyramidal neurons, in a similar way to that observed when COREST or LSD1 expressions are decreased. Diminished SFPQ expression also associates to decreased proliferation of progenitor cells, while it does not affect the acquisition of neuronal fate. These results are compatible with the idea that SFPQ, plays an important role regulating proliferation and migration during the development of the cerebral cortex.

1. Introduction

The generation of pyramidal glutamatergic neurons during the development of the cerebral cortex proceeds through a sequence of steps that involves the exit from cell cycle of neural progenitors, the suppression of progenitor cell identity, the initiation of neuronal differentiation and radial migration towards the cortical plate of newborn neurons (Takahashi et al., 1999; Noctor et al., 2004; Kriegstein et al., 2006). The adequate execution of the process requires a fine regulation of gene expression that results from the coordinated action of both transcriptional and post-transcriptional mechanisms (Molyneaux et al., 2007; Darnell, 2013). Epigenetic regulation plays an important role in the control of spatial and temporal patterns of gene expression during neural development (Riccio, 2010). Epigenetic modifications of chromatin are carried out by multicomponent coactivator or corepressor complexes which are selectively recruited towards specific regulatory elements in the DNA by transcription factors, resulting in covalent modifications of the chromatin surrounding the transcription factor binding site.

Changes of the acetylation and methylation status of histones are important mechanisms of epigenetic regulation. COREST (also known as RCOR1) and the histone demethylase LSD1 (also known as KDM1A) are key regulators of these covalent modifications of the chromatin and have been shown to play a critical role during cerebral cortex development (You et al., 2001; Shi et al., 2004; Fuentes et al., 2012; Lopez et al., 2016). The unveiling of how these proteins assemble in complexes, how their function is targeted to particular domains of chromatin and which genes they regulate is important to understand the coordination of processes that underlie the complex genesis of the multilayered mammalian cerebral cortex. COREST is a multidomain transcriptional corepressor that regulates gene expression through its ability to recruit and form complexes with chromatin-modifying proteins including class I histone deacetylases (HDAC1/2) (Humphrey et al., 2001; You et al., 2001; Hakimi et al., 2002) and LSD1 (Shi et al., 2005; Foster et al.,

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2010). COREST, first identified as a corepressor of REST (also known as NRSF) (Andrés et al., 1999), interacts with LDS1 and enables its histone H3 demethylase activity (Forneris et al., 2007; Yang et al., 2006). In addition, COREST inhibits LSD1 proteosomal degradation (Lee et al., 2005; Shi et al., 2005). Conversely, conditional deletion of LSD1 from embryonic stem cells leads to a reduction of COREST protein (Foster et al., 2010), suggesting a close functional relationship between these proteins. shRNA mediated knock-down of COREST or LSD1 during cortex development leads to a transitory migratory delay of newborn pyramidal neurons that is accompanied by an increase in the number of progenitor cells (Fuentes et al., 2012; Lopez et al., 2016). The function of COREST/LSD1 complex during cortical development appears to be independent of the function of REST, as REST depleted neurons migrate normally to the cortical plate (Fuentes et al., 2012). These observations are in agreement with previous studies showing that COREST may operate independently of REST in the regulation of specific gene networks responsible for inducing and maintaining neuronal identity (Ballas et al., 2005; Abrajano et al., 2009). Thus, it is reasonable to propose that the function of the complex COREST/LSD1 during development may be associated to other unidentified transcriptional regulators.

We conducted a study aimed to identify proteins associated to COREST/LSD1 which could contribute to direct and complement the function of this complex in the context of cerebral cortex development. In this article we communicate that the multi-functional protein SFPQ (splicing factor proline-glutamine rich) is associated to LSD1 in the developing cerebral cortex. Decreasing the levels of SFPQ in the developing mouse cerebral cortex *in vivo* leads to marked defects in the radial migration of pyramidal neurons without affecting neuronal differentiation, as well as to changes in the dynamics of progenitor population, in a way similar to the one observed with the depletion of LSD1 (Fuentes et al., 2012; Lopez et al., 2016).

2. Materials and methods

2.1. Animals

Animal studies described here were carried out in the CF-1 strain mice and followed NIH (USA) and CONICYT (Chile) guidelines. The institutional Bioethics Committee (Universidad de Chile) approved all animal protocols (CBA, 0474).

2.2. Plasmids

pCAGIG-COREST-HA and pCAGIG-LSD1-HA were previously engineered in our laboratory (Fuentes et al., 2012; Lopez et al., 2016). pMYC-PSF-WT (Rosonina et al., 2005) was obtained from Addgene (ID# 35183). pLV-hU6-mPKG-green pre designed shRNA kit (cat# RNAi-B06-NM_023603.3) was purchased by Biosettia (San Diego, CA). It includes a non-sense ("shControl") and four SFPQ shRNAs expressing plasmids (sh-NM-023603-1095; sh-NM-023603-1332; sh-NM-023603-1433; sh-NM-023603-1542). The sequences of the shRNAs used were:

"shControl":

AAAAGCTACACTATCGAGCAATTTTGGATCCAAAATTGCTC GATAGTGTAGC

sh-NM-023603-1095 (sh1SFPQ):

AAAAGGGTTCATTAAGCTTGAATTTGGATCCAAATTCAAG CTTAATGAACCC

sh-NM-023603-1332 (sh2SFPQ):

AAAAGGCATTGTTGAGTTTGCTTTTGGATCCAAAAGCAAAC TCAACAATGCC

sh-NM-023603-1433 (sh3SFPQ):

AAAAGGAACCACTTGAACAGTTATTGGATCCAATAACTGTTC AAGTGGTTCC

sh-NM-023603-1542 (sh4SFPQ):

AAAAGGCACATTTGAGTATGAATTTGGATCCAAATTCATACTC AAATGTGCC

2.3. Isolation of LSD1 interacting proteins

Mouse E14.5 embryonic brain cortices were homogenized by sonication in RIPA buffer (50 mM Tris, pH 7,5; 150 mM NaCl; 1 mM EDTA; 0,25% Deoxicolate; 1% Nonidet P-40) supplemented with a protease inhibitor cocktail (Roche, cat# 04693116001). Homogenates were centrifuged at 17,000*g* for 20 min at 4 °C to remove debris. Precleared embryonic cortex protein extract (3 mg in 1 ml) was incubated with rabbit polyclonal anti LSD1 (Abcam, cat# ab17721) or normal rabbit IgG (Santa Cruz, cat# sc-2027) cross-linked to protein G-agarose (KPL, cat# 223-51-00) overnight at 4 °C. Protein G-agarose cross-link was performed with dimethyl pimelimidate (Thermo Scientific, cat# 21666) according to the manufacturer's instructions. Eluates were run in polyacrilamide gels on PROTEAN® II xi Cell system and silver stained (Shevchenko et al., 1996). Protein enriched bands in anti-LSD1 immunoprecipitates were selected for mass spectrometry.

2.4. Mass spectrometry

Samples were proteolyzed with trypsin, separated by high-pressure chromatography (EASY-nLC II, Proxeon, Thermo Scientific) and detected by mass spectrometry (LTQ Velos, Thermos Scientific). Data were processed with MS Cleaner 2.0 (http://mendel.bii-sg.org/mass-spectrometry/MSCleaner-2.0/) and analyzed with MASCOT Server 2.1.03 version (Matrix Science, UK) and local server PEAKS Studio 6.0, using the SwissProt (2016) database, considering *Mus musculus* taxonomy, trypsin proteolysis, one missed cleavages, methionine oxidation, monoisotopc mass, mass tolerance 0.8 Da, fragmentation tolerance 0.8 Da, peptide charge +1, +2 and +3. We used a decoy database to eliminate false positives. Only statistically significant proteins obtained from both servers were taken for further analysis. Identified proteins were analyzed and classified according their biological functions with DAVID (http://david.abcc.ncifcrf.gov).

2.5. Cell culture and transfection

The Neuro-2a (N2a) cell line was obtained from ATCC and grown under standard conditions in DMEM (Invitrogen) medium supplemented with fetal bovine serum (Hyclone) to a final concentration of 10%. For transfection experiments, 10^5 cells were grown in 35 mm dishes and transfected using Lipofectamine 2000 reagent (Life Technologies) according to the manufacturer's instructions. After 72 h of transfection, cell cultures were lysed to evaluate SFPQ expression by Western blot or to perform co-immunoprecipitation assays.

2.6. Co-immunoprecipitation assays

N2a cell cultures were non-transfected (control) or transfected with: pCAGIG-LSD1-HA; pCAGIG-COREST-HA or pMYC-PSF-WT (Rosonina et al., 2005). Whole cell lysates were prepared by sonication in RIPA buffer and incubated with 2 μg rabbit polyclonal anti HA (Abcam, cat# ab9110) or mouse monoclonal anti MYC (Sigma, cat# M4439) overnight at 4 °C. Co-immunoprecipitation was performed with Pierce Classic Magnetic IP/Co-IP kit (cat# 88804) according to the manufacturer's instructions. Cell lysates and immunoprecipitates were analyzed by Western blot. To analyze the interaction between LSD1 and SFPQ in the developing