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NCoR/SMRT complex regulates gene expression in the brain

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science
in
Biology
by
Yong Ryoul Kim

Committee in charge:

Professor Michael G. Rosenfeld, Chair
Professor James Kadonaga, Co-Chair
Professor Brenda Bloodgood

2017

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2017

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ABSTRACT OF THE THESIS

NCoR/SMRT complex regulate gene expression in the brain

by

Yong Ryoul Kim
Master of Science in Biology
University of California, San Diego, 2017

Professor Michael G Rosenfeld, Chair
Professor James Kadonaga, Co-Chair

Nuclear receptor co-repressors NCoR and SMRT are transcriptional regulatory proteins that interact with different transcription factors and epigenetic modifiers. Here, we study how NCoR/SMRT co-repressor complex regulates neuronal gene expression. In the lab, conditional knock-out mice have been developed to delete the NCoR and SMRT genes specifically in excitatory neurons of the postnatal forebrain. The conditional deletion of these genes is necessary because the traditional null mice die during embryonic development. Preliminary behavioral data show that the genetic deletion of both genes leads to stress-related behaviors. Hence, we hypothesize that NCoR/SMRT complex regulates the genomic action of glucocorticoid receptor (GR), a nuclear hormone receptor that functions as a cellular sensor for stress and it is known to interact with the NCoR/SMRT complex. To identify GR target genes regulated by NCoR/SMRT complex in

the brain, we used NCoR/SMRT double knock-out mice and a transcriptomic approach known as Global Run-On sequencing. Subsequent bioinformatics analysis of sequencing data showed that the expression of hundreds of genes is altered upon genetic deletion of NCoR and SMRT. Moreover, biological processes linked to glucocorticoid signaling, such as immune response and circadian rhythm, were found to be dysregulated in the absence of NCoR and SMRT. These results provide evidence that NCoR/SMRT complex mediates the transcriptional regulation of key molecular pathways linked to the function of glucocorticoids in the brain.

Introduction

Throughout life, neurons in the central nervous system continuously make appropriate changes in response to internal and external stimuli, such as level of hormones and stress. The ability of neurons to adapt relies in part on changes in gene expression. Advances in sequencing technologies coupled with molecular techniques have improved our understanding of how the genome elaborates different information to regulate gene expression (Telese et al., 2013). Among these techniques, Global Run-On sequencing (GRO-seq) allows measurement of nascent transcription catalyzed by RNA polymerase in real-time (Gardini A., 2017). The GRO-seq is a transcriptomic assay based on the labeling of newly synthetized transcripts with a modified ribonucleotide (BrdUTP). BrdUTP can be detected with anti-BrdUTP antibodies and can be used for the isolation of nascent transcripts labeled in vitro. These RNA molecules are then purified and used for the preparation of the sequencing library. We applied this methodology to identify the gene expression programs regulated by the NCoR/SMRT in the brain.

NCoR and SMRT are structurally related proteins that function that negatively regulate gene expression of target genes. The corepressors form a multi-protein complex with other repressor proteins, including TBL1, TBLR, GPS2 and HDAC3 (Perissi et al.,2010). Previous work showed that NCoR/SMRT corepressor complex occupies distal enhancer regions required for the activation of Reelin target genes, which are involved in mechanisms of learning and memory (Telese et al., 2015). In the absence of Reelin, the NCoR/SMRT complex suppresses the activation of target genes by recruiting histone deacetylases, such as HDAC4. Moreover, various research groups have shown that NCoR/SMRT complex can interact with glucocorticoid receptor (GR) (Schulz et al.,2002). Glucocorticoids are a class of steroid hormones that bind to glucocorticoid receptors and are produced by the adrenal glands to regulate numerous endogenous functions and to respond to external stressors (Barnes.,2014). Glucocorticoids levels are modulated by the circadian rhythm and are increase by stress (Harris et al 2013). Notably, stress has the ability to modulate cognitive abilities, such as memory formation (Finsterwald et al.,2014).

Therefore, we hypothesize that NCoR and SMRT, by modulating GR transcriptional activity, are involved in how cognition is affected in presence of increased levels of stress.

To address this question, I took advantage of available mice model in the lab, namely the NCoR/SMRT conditional double knock-out (dKO) mice. These mice lack the expression of NCoR and SMRT genes in Calcium/Calmodulin Dependent Protein Kinase II Alpha(Camk2a)-positive excitatory neurons. Simultaneously, these neurons express the nuclear envelope protein Sun1 fused with the green fluorescent protein (GFP). This system allows the immune-isolation of nuclei from the brain using antibodies that recognizes GFP on the surface of the nuclear envelope (Mo et al., 2015). Nuclei isolated from the brain of dKO mice were used for transcriptomic analysis by GRO-seq. Finally, we report that the genetic deletion of NCOR and SMRT in excitatory neurons leads to the transcriptional dysregulation of hundreds of genes. Interestingly, these genes are associated with mechanisms of immune function and circadian rhythm, supporting our hypothesis that NCoR/SMRT might mediate glucocorticoids signaling in the brain.

Materials and Methods

Genotype

DNA was extracted from tail biopsies in 50mM NaOH. 2ul of extracted DNA was used as template for PCR. For each gene (NCoR, SMRT, Sun1) or transgene (Cre), we used specific primer pairs. The following thermocycler settings were used for 35 cycles: DNA denaturation was performed at 95C, primer annealing temperature at 60C and extension temperature at 72C. Each PCR reaction was then analyzed by gel electrophoresis. Sequences of forward and reverse primer pairs used are as follow:

NCoR(F:AGGTGGAATCACAGAAAGCTGACGC,R:AACTAAGGCACAGGAAGGTACAGGG), SMRT(F: GCAGCCATGCTATGTCCTCT, R: CCCGAGACACCATCTGCTAT), LACZ(F: ATATTGAAACCCACGGCATGG,R: TTTGATGGACCATTTCGGCAC),

Sun1(F: GTT ATG TAA CGC GGA ACT CC, R: CAT AGT CTA ACT CGC GAC ACT G,R: GCA CTT GCT CTC CCA AAG TC),CRE(F: TGAGGTTCGCAAGAACCTGATGGA, R: GCCGCATAACCAGTGAAACAGCAT)

Western Blot

Isolated nuclei from brain tissue are lysed in RIPA buffer(150mM NaCl, 20mM Tris ph=7.4,1mM EDTA, 1.5mM MgCl2, 1% IGEPAL). Extracted proteins were analyzed by SDS-PAGE gel electrophoresis. After denaturation of proteins for 5 minute at 95C, proteins were resolved on a 3-8% Tris Acetate gel for 90 minutes at 200V. After gel electrophoresis, proteins were transferred to a PVDF membrane for 1 hour at 20V. After blocking in blocking buffer, the membrane was incubated over night with the primary antibody anti-NCoR and one hour with the secondary antibody conjugated with the horseradish peroxidase (**HRP**). Chemiluminescence was used to visualize proteins.

Sample Preparation and Nuclei Isolation

Protein G covalently bound to magnetic beads were conjugated with 4ug of GFP antibody (Invitrogen G10362). Dissected brain tissue (neocortex) from three control and

three double-KO mice was homogenized using a motorized homogenizer in homogenization bugger (HB). NP40 was added at a final concentration of 0.5% to release the nuclei. Nuclei were recovered by centrifugation and incubated with GFP antibody-bead complex. After magnetic labeling, the nuclei were stained with a DNA dye (DAPI) and counted using a cell counting software of the Keyence microscope.

GRO-seq Libraries Preparation

5 million nuclei were isolated from the neocortex of dissected brains. The nuclei were incubated in nuclear run on (NRO) reaction buffer at 30C for 5 minute. NRO-RNA was then extracted using Trizol LS and RNA was precipitated using isopropanol. The purified RNA was subjected to an hydrolysis step by incubation with 1M NaOH for 30 minutes. Buffer exchange was performed with biospin p-30 RNase free columns. Agarose beads conjugated with the anti-BrdU antibody were washed and then blocked for 45 minutes with blocking buffer. Binding of BrdU-agarose beads and purified NRO-RNA was carried out at 4C for 4hours. After washing the BrdU-beads/RNA complex with low- and high-salt containing washing buffers, Trizol was used to purify the immunoprecipitated RNA. Purified RNA was used for sequencing library preparation following instructions of the NEB small library preparation kit(E7330S). The library preparation workflow includes 3-prime and 5'-prime adapters ligation, reverse transcription and PCR amplification. The PCR amplification was carried out with low (12) and high (15) cycles numbers to amplify the optimal amount of library. The DNA library was selected for fragments sizes ranging between 175bp to 400bp, by agarose gel extraction.

Bioinformatic analysis

The bioinformatics analysis was performed by Dr Telese using an available pipeline in the lab.

Results

In this study, I used genetically modified mouse lines that were previously generated by Dr Telese in Dr Rosenfeld lab. The control mice express normal levels of the NCoR and SMRT genes in the Camk2a-positive excitatory neurons of the postnatal forebrain. The Control mice also express the nuclear envelop protein Sun1 as a GFP-fused protein in excitatory neurons. Conversely, the double-knockout mice do not express NCoR and SMRT genes, but still express the Sun1-GFP. To validate the genotype of these mice, I used polymerase chain reaction to amplify the mutated genomic loci that were visualized by agarose gel electrophoresis. In figure 1, representative results of the genotyping protocol are shown. As shown in Figure 1A, in Control mice, the genotyping result for NCoR and SMRT gene is the same as in WT mice, while the genotyping result for Sun1 and CRE genes are modified. In Figure 1B, the genotype of a double-KO mouse is compared to a WT mouse. All the loci are genetically modified, as shown by the PCR results. These genotypes were selected for our experiment in which Sun1 is expressed as a GFP-fused protein in both Control and dKO mice, while NCoR/SMRT expression is abolished only in the dKO mice.

Because the double knock out condition is cell type specific, we need to sort the excitatory neurons from other cell types of the heterogeneous brain tissue. We take advantage of the Sun1 construct that leads to the expression of GFP at the surface of the nuclear envelop in excitatory neurons. Therefore, we isolated GFP-positive nuclei from Control and dKO brains using anti-GFP antibodies conjugated to magnetic beads. Upon magnetic labeling, we achieved significant enrichment of GFP-positive nuclei (85%) (Figure 2). The enrichment is approximately two folds compared to the pre-labeled nuclei. To further validate our experimental system, we examined NCoR protein expression by western blot. As expected, NCoR signal is intense in the nuclei isolated from the control sample, but not in the dKO sample, which shows a complete loss of NCoR protein (Figure 3). This result demonstrates that the conditional deletion of NCoR is complete and that our immunoisolation protocol is efficient. Isolated nuclei from excitatory neurons were

utilized to perform the in vitro transcription (run-on reaction) using a modified ribonucleotide (Br-UTP). The labeled transcripts were isolated with antibodies anti-Br-UTP. Purified RNA molecules were used for the preparation of the sequencing library. The GRO-seq library includes different steps, such as 5` adaptor ligation, 3`adaptor ligation, and reverse transcription to generate the cDNA library. Lastly, the cDNA is amplified by PCR with primers containing unique sequences utilized by the Illumina sequencer. Figure 4 shows the final product of amplified DNA and optimization of PCR cycle number to determine ideal amount of library for sequencing. The library obtained with 15 rounds of amplification showed a higher content of DNA and was used for sequencing (Figure 4). The library was sequenced on the Illumina HiSeq400 at the UCSD sequencing core facility. Based on the bioinformatics analysis performed by Dr Telese, we found that the genetic deletion of NCoR and SMRT in excitatory neurons affects the expression of 198 genes. More specifically, 148 genes were up-regulated and 50 genes were down-regulated (Figure 5). The observation that most genes were up-regulated in absence of NCoR and SMRT is not surprising given that this complex is known to repress transcription. Results obtained by performing a gene ontology (GO) analysis suggest that the differentially expressed genes are associated with specific biological processes, as shown in Table 1. Most notably, immune function, inflammatory response, and circadian gene regulation are the most enriched biological processes that are associated with the differentially expressed genes. This is particularly intriguing given that glucocorticoid receptor is involved in regulation of these pathways (Coutinho et al.,2011; Chung et al.,2011). Hence, this result suggests that NCoR/SMRT complex regulates gene expression in excitatory neurons and might affect the function of glucocorticoids signaling.

Discussion

With the present study, we expand our understanding of mechanisms of transcriptional regulation in neurons by taking advantage of cutting-edge genomic technologies. First, we validate a novel genetic mouse model based on the conditional deletion of NCoR and SMRT genes in excitatory neurons of the postnatal forebrain. Then, we provide clear evidence that Sun1-GFP transgenic mice can be efficiently utilized to examine the transcriptome of specific cell-types in the brain. We used GRO-seq to uncover the role of NCoR and SMRT in the regulation of specific gene expression programs. Furthermore, we begin to understand the biological pathways regulated by this transcriptional complex based on the gene ontology analysis of the GRO-seq results. Particularly interesting is the identification of genes associated with immune response and circadian rhythm, both functions known to be modulated by glucocorticoid receptor. In fact, it is well established that the circadian rhythm is regulated by the action of glucocorticoid receptor, and that glucocorticoids can induce immune suppression (Yick-Lun So.,2009). While our data suggest a cross talk between NCoR/SMRT complex and GR-regulated transcriptional pathways in neurons, exact molecular mechanisms explaining this interaction remain to be explored. It has been previously shown that NCoR/SMRT complex is required for the recruitment of GR to distal enhancer elements occupied by the estrogen receptor in breast cancer cells (Yang et al.,2017). Therefore, it will be of particular interest to elucidate whether NCoR/SMRT complex uses a similar mechanism in neuronal cells to regulate GR function in response to stress hormones. These studies open up new avenues of research that have the potential to elucidate to what extent NCoR/SMRT complex suppresses immune function in the brain. By combining cell type-specific nuclei isolation, genome wide transcription profiling, and gene ontology analysis, we were able to identify novel transcriptional pathways that are relevant to critical brain functions, such as circadian rhythm and immune function.

Figures

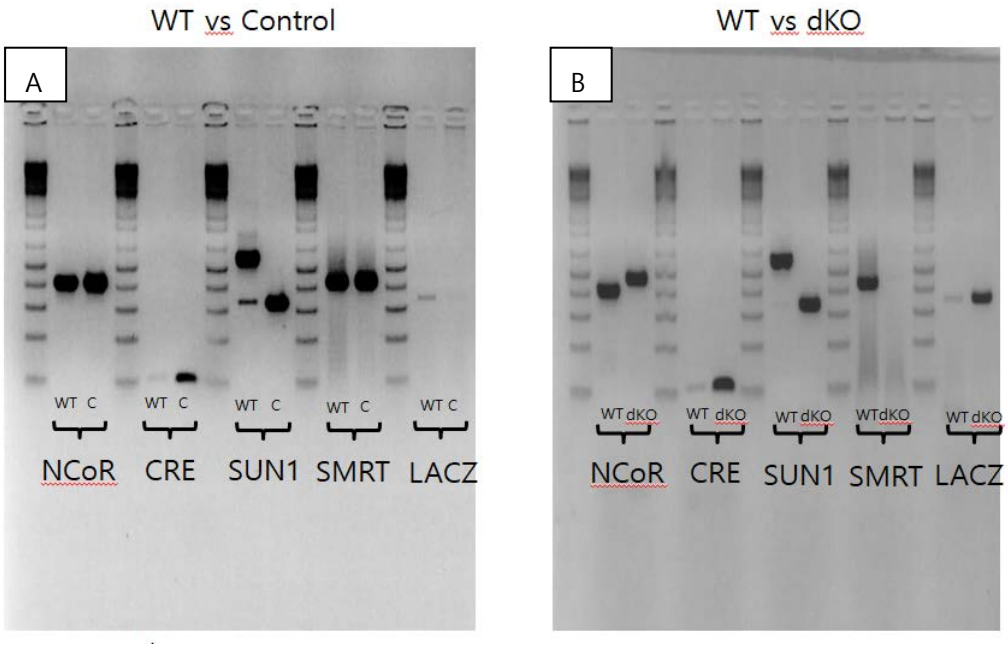

Figure 1. Genotype

Validation of Control (Sun1-GFP) and NCoR/SMRT double knock-out (dKO) (Sun1-GFP) mice. Comparison between wild type (WT), Control (Sun1-GFP) or dKO mice is shown. 1A) PCR products for different genes (NCoR, CRE, Sun1, SMRT and Lacz) are visualized by gel electrophoresis for WT and Control mice 1B) PCR products for different genes (NCoR, CRE, Sun1, SMRT and Lacz) are visualized by gel electrophoresis for WT and dKO mouse.

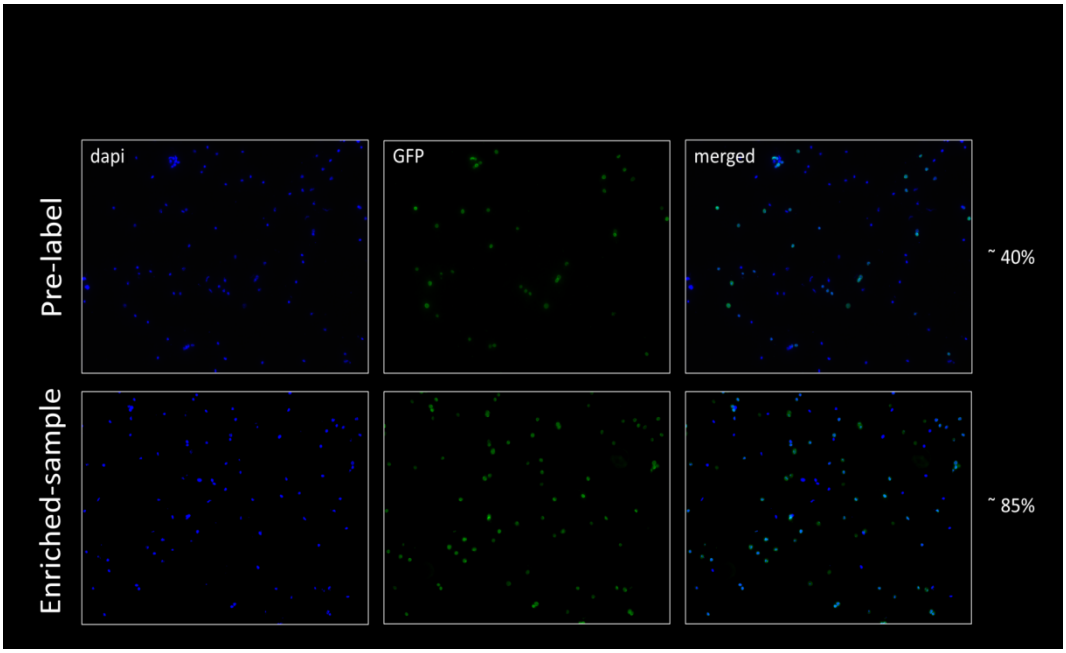

Figure 2. Nuclei Visualization

Immunopurification of GFP-positive nuclei from Sun1-GFP mice visualized at the fluorescent microscope. The top panel (from left to right) shows fluorescent signal for DNA dye (DAPI), green fluorescent protein (GFP) and merged signal from pre-labeled nuclei, before immunoprecipitation with anti-GFP antibody. 40% of nuclei are GFP-positive. The bottom panel shows the nuclei after immunopurification with GFP-antibody. 85% of nuclei are GFP-positive (>2 -fold enrichment compared to pre-label sample).

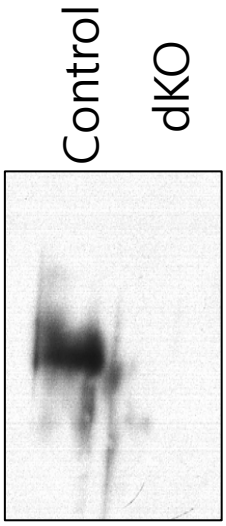

Figure 3. Western Blot

Validation of dKO mice by western blot. Western blot image of NCoR expression in immonoisolated nuclei from brain tissue of Control and dKO mice. NCoR protein expression is completely abolished in dKO excitatory neurons.

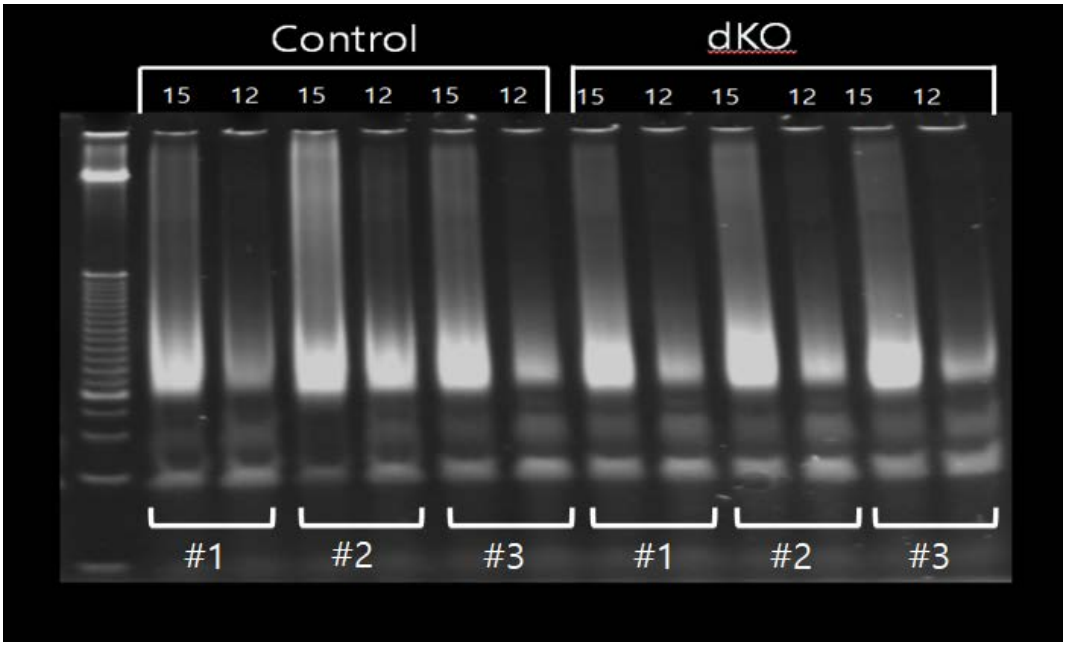

Figure 4. GRO-seq Library

PCR amplification of GRO-seq library. RNA isolated from three Control and dKO mie was used for GRO-seq library preparation. DNA library were amplified for 12 and 15 cycles.

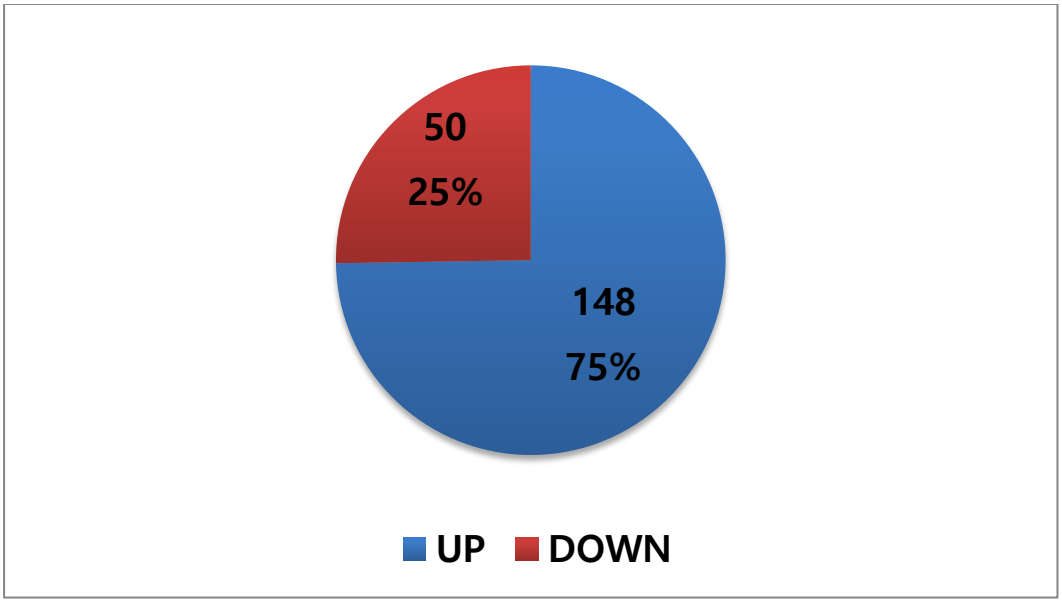

Figure 5. Bioinformatics analysis

Pie chart showing genes differentially regulated by deletion of NCoR/SMRT proteins assessed by GRO-seq analysis in excitatory neurons of cortex.

Table 1. Gene Ontology

Gene Ontology analysis of differentially regulated genes identified by GRO-seq. Most significantly enrich Gene ontology annotations are shown.

GO TERM	P-Value
immune system process	7.14E-07
inflammatory response	6.00E-06
microglial cell activation	4.17E-04
circadian regulation of gene expression	1.64E-03
toll-like receptor signaling pathway	8.57E-03
positive regulation of transcription	8.64E-03

Reference

1. Telese, Francesca, Amir Gamliel, Dorota Skowronska-Krawczyk, Ivan Garcia-Bassets, Michael G. Rosenfeld. “seqing Insights into the Epigenetics of Neuronal Gene Regulation.”Neuron,vol.77,no.4,2013,pp.606–623., doi:10.1016/j.neuron.2013.01.034.
2. Gardini, Alessandro. “Global Run-On Sequencing (GRO-Seq).” Methods in Molecular Biology Enhancer RNAs, 2016, pp. 111–120., doi:10.1007/978-1-4939-4035-6_9.
3. Perissi V, Jepsen K, Glass CK, Rosenfeld MG. “Deconstructing Repression: Evolving Models of Co-Repressor Action.” Nature Reviews Genetics, vol. 11, no. 2, 2010, pp. 109–123., doi:10.1038/nrg2736.
4. Telese F, Ma Q, Perez PM, Notani D, Oh S, Li W, Comoletti D, Ohgi KA, Taylor H, Rosenfeld MG. “LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and Memory Formation.” Neuron, vol. 86, no. 3, 2015, pp. 696–710., doi:10.1016/j.neuron.2015.03.033.
5. Schulz M, Eggert M, Baniahmad A, Dostert A, Heinzel T, Renkawitz R. “RU486-Induced Glucocorticoid Receptor Agonism Is Controlled by the Receptor N Terminus and by Corepressor Binding.” Journal of Biological Chemistry, vol. 277, no. 29, 2002, pp. 26238–26243., doi:10.1074/jbc.m203268200.
6. Barens PJ,”Glucocorticoid” Chem Immunol Allergy. 2014;100:311-6. doi: 10.1159/000359984.
7. Finsterwald, Charles, and Cristina M. Alberini. “Stress and Glucocorticoid Receptor-Dependent Mechanisms in Long-Term Memory: From Adaptive Responses to Psychopathologies.” Neurobiology of Learning and Memory, vol. 112, 2014, pp. 17–29., doi:10.1016/j.nlm.2013.09.017.
8. Harris, A.p, Holmes MC,de Kloet ER, Chapman KE, Seckl JR. “Mineralocorticoid and Glucocorticoid Receptor Balance in Control of HPA Axis and Behaviour.”Psychoneuroendocrinology, vol. 38, no. 5, 2013, pp. 648–658., doi:10.1016/j.psyneuen.2012.08.007.
9. Alisa Mo, Eran A. Mukamel, Fred P. Davis, Chongyuan Luo, Gilbert L. Henry, Serge Picard, Mark A. Urich, Joseph R. Nery, Terrence J. Sejnowski, Ryan Lister, Sean R. Eddy, Joseph R. Ecker, Joseph R. Ecker, Jeremy Nathans. “Epigenomic Signatures of Neuronal Diversity in the Mammalian Brain.” Neuron, vol. 86, no. 6, 2015, pp. 1369–1384., doi:10.1016/j.neuron.2015.05.018.
10. Coutinho, Agnes E., and Karen E. Chapman. “The Anti-Inflammatory and Immunosuppressive Effects of Glucocorticoids, Recent Developments and Mechanistic Insights.” Molecular and Cellular Endocrinology, vol. 335, no. 1, 2011, pp. 2–13., doi:10.1016/j.mce.2010.04.005.
11. Chung, Sooyoung, Son GH, Kim K. “Circadian Rhythm of Adrenal Glucocorticoid: Its Regulation and Clinical Implications.” Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease, vol. 1812, no. 5, 2011, pp. 581–591., doi:10.1016/j.bbadis.2011.02.003.
12. So, A. Y.-L., Teresita U. Bernal, Marlisa L. Pillsbury, Keith R. Yamamoto and Brian J. Feldman. “Glucocorticoid Regulation of the Circadian Clock Modulates Glucose Homeostasis.” Proceedings of the National Academy of Sciences, vol. 106, no. 41, May 2009, pp. 17582–17587., doi:10.1073/pnas.0909733106.
13. Yang F, Ma Q, Liu Z, Li W, Tan Y, Jin C, Ma W, Hu Y, Shen J, Ohgi KA, Telese F, Liu W, Rosenfeld MG. “Glucocorticoid Receptor:MegaTrans Switching Mediates the Repression of an ERÎ±-Regulated Transcriptional Program.” Molecular Cell, vol. 66, no. 3, 2017, doi:10.1016/j.molcel.2017.03.019.