ELUCIDATION OF THE GENE REGULATORY NETWORK RELATED TO SPINAL MUSCULAR ATROPHY

K. HUANG [1, 2], Y. HUANG [1], X. FANG [1], Z. ZHANG [1], Y. WANG [2], D. PENG [1, 3]

[1] Department of Medical Genetics, Changde Hospital, Xiangya School of Medicine, Central South University (The first people's hospital of Changde city) Hunan Changde, 415000 China

[2] Hunan Provincial Key Laboratory for Molecular Immunity Technology of Aquatic Animal Diseases, College of Life and Environmental Sciences, Hunan University of Arts and Science, Hunan Changde, 415000 China.

[3] Affiliated Hospital of Changde City, University of South China, Hengyang, 421000 China

E-mail: huangkerui@huas.edu.cn, 1553711350@qq.com, hyz20202022@163.com, fphcfxx@163.com, zhangzhaoxia0322@163.com, wy2015@huas.edu.cn, dasan0988@126.com

Correspondence: HUANG K., HUANG Y., e-mail: hyz20202022@163.com, dasan0988@126.com

Spinal muscular atrophy (SMA) is an autosomal recessive heritable disorder leading to abnormalities and dysfunction of alpha motor neurons, paralysis, and eventual death due to respiratory failure. However, the gene regulatory mechanism related to SMA is still not completely clear. Here, we constructed the gene regulatory network of SMA, in which several SMA-related genes and transcription factors played important roles. In the process, 6544 differentially expressed genes (DEGs) associated with SMA were used and the enrichment analysis and gene regulatory network construction using machine learning was performed. The result showed that, firstly, p53 signaling and DNA replication are closely related to SMA. Then, there is a huge and complicated regulatory network guided by SMA, in which transcription factor SNAPC2, MZF1, and ZNF711 interacted closely with SMA-related genes (SMN1, SMN2) and played key roles in regulating genes of p53 signaling, DNA replication and other SMA-related GO (Gene Ontology) terms. The transcriptome data was well verified through real-time fluorescence quantitative PCR (RT-qPCR) using the peripheral blood of spinal muscular atrophy patients. Our study revealed the complicated gene regulation network of SMA, and uncover several important SMA-related genes, which it deepens our understanding of SMA-related regulatory mechanisms.

Key words: *Spinal muscular atrophy, transcription factors, machine learning, p53 signaling.*

ДОСЛІДЖЕННЯ ГЕННОЇ РЕГУЛЯТОРНОЇ МЕРЕЖІ, ПОВ'ЯЗАНОЇ ЗІ СПІНАЛЬНОЮ М'ЯЗОВОЮ АТРОФІЄЮ

Спінальна м'язова атрофія (СМА) — це аутосомно-ресецивний спадковий розлад, що призводить до патології і дисфункції альфа-моторних нейронів, паралічу і зрештою смерті через дихальну недостатність. Однак механізм генної регуляції, пов'язаний зі СМА, все ще не до кінця зрозумілий. У цьому дослідженні ми сконструювали генну регуляторну мережу СМА, в якій кілька пов'язаних із СМА генів і транскрипційних факторів відіграють важливу роль. У процесі роботи було використано 6544 диференційовано експресованих генів (DEG), пов'язаних із СМА, і проведено аналіз збагачення та побудову генної регуляторної мережі з використанням машинного навчання. Результати продемонстрували, що, по-перше, сигналізація p53 і реплікація ДНК тісно пов'язані зі СМА. По-друге, існує величезна і складна регуляторна мережа, керована СМА, в якій транскрипційні фактори *SNAPC2, MZF1* і *ZNF711* тісно взаємодіють з генами, пов'язаними з СМА (*SMN1, SMN2*), і відіграють ключову роль у регуляції генів сигналізації p53, реплікації ДНК та інших пов'язаних із СМА термінів GO (Gene Ontology). Транскриптомні дані були верифіковані за допомогою флуоресцентної кількісної ПЛР у реальному часі (RT-qPCR) з використанням периферичної крові пацієнтів зі спінальною м'язовою атрофією. Наше дослідження виявило складну мережу генної регуляції СМА та відкрило кілька важливих генів, пов'язаних із СМА, що поглиблює наше розуміння регуляторних механізмів, пов'язаних із СМА.

Ключові слова: спінальна м'язова атрофія, транскрипційні фактори, машинне навчання, сигналізація p53.

REFERENCES

Ahmad S, Wang Y, Shaik GM, Burghes AH, Gangwani L (2012) The zinc finger protein ZPR1 is a potential modifier of spinal muscular atrophy. Hum Mol Genet 21:2745–2758. https://doi.org/10.1093/hmg/dds102

Ahn E-J, Yum M-S, Kim E-H, Yoo H-W, Lee BH, Kim G-H, Ko T-S (2017) Genotype-phenotype correlation of SMN1 and NAIP deletions in Korean patients with spinal muscular atrophy. J Clin Neurology 13:27–31. https://doi.org/10.3988/jcn.2017.13.1.27

Arnold WD, Kassar D, Kissel JT (2015) Spinal muscular atrophy: diagnosis and management in a new therapeutic era. Muscle Nerve 51:157–167. https://doi.org/10.1002/mus.24497

Calucho M, Bernal S, Al AsL, March F, Vencesl A, Rodr

Guez-Álvarez FJ, Aller E, Fern Ndez RM, Borrego S, Mill NJM (2018) Correlation between SMA type and SMN2 copy number revisited: an analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. Neuromuscul Disor. 28:208–215. https://doi.org/10.1016/j.nmd.2018.01.003

Carr A, Empey C (2016) Review of spinal muscular atrophy (SMA) for prenatal and pediatric genetic counselors. J Genet Counsel 25:32–43. https://doi.org/10.1007/s10897-015-9859-z

Coovert DD. Le TT, Mcandrew PE, Strasswimmer J, Crawford TO, Mendell JR, Coulson SE, Androphy EJ, Prior TW, Burghes AH (1997) The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 6:1205–1214. https://doi.org/10.1007/s00018-021-03819-5

Cuartas J, Gangwani L (2022) R-loop Mediated DNA Damage and Impaired DNA Repair in Spinal Muscular Atrophy. Front Cell Neurosci 16. https://doi.org/10.3389/fncel.2022.826608

Fallini C, Zhang H, Su Y, Silani V, Singer RH, Rossoll W, Bassell GJ (2011) The survival of motor neuron (SMN) protein interacts with the mRNA-binding protein HuD and regulates localization of poly (A) mRNA in primary motor neuron axons. J Neurosci 31:3914,3925. https://doi.org/10.1523/JNEUROSCI.3631-10.2011

Groen EJ, Talbot K, Gillingwater TH (2018) Advances in therapy for spinal muscular atrophy: promises and challenges. Nat Rev Neurol 14:214–224. https://doi.org/10.1038/nrneurol.2018.4

Huang K, Zhou S, Shen K, Zhou Y, Wang F, Jiang X (2020) Elucidation of the miR164c-guided gene/protein interaction network controlling seed vigor in rice. Front Plant Sci 11:589005. https://doi.org/10.3389/fpls.2020.589005

Huang Z (2009) Molecular regulation of neuronal migration during neocortical development. Mol Cell Neurosci 42:11–22. https://doi.org/10.1016/j.mcn.2009.06.003

Ihmels J, Friedlander G, Bergmann S, Sarig O, Ziv Y, Barkai N (2002) Revealing modular organization in the yeast transcriptional network. Nat Genet 31:370–377. https://doi.org/10.1038/ng941

Jangi M, Fleet C, Cullen P, Gupta SV, Mekhoubad S, Chiao E, Allaire N, Bennett CF, Rigo F, Krainer AR (2017) SMN deficiency in severe models of spinal muscular atrophy causes widespread intron retention and DNA damage. Proc Nat Acad Sci 114:E2347-E2356. https://doi.org/10.1073/pnas.161318111

Jarrous N (2017) Roles of RNase P and its subunits. Trends Genet 33:594–603. https://doi.org/10.1016/j.tig.2017.06.006

Kannan A, Jiang X, He L, Ahmad S, Gangwani L (2020) ZPR1 prevents R-loop accumulation, upregulates SMN2 expression and rescues spinal muscular atrophy. Brain 143:69–93. https://doi.org/10.1093/brain/awz373

Kim EK, Choi E-J (2017) SMN1 functions as a novel inhibitor for TRAF6-mediated NF-κB signaling. Biochimt Biophys Acta (BBA)-Molecular Cell Res 1864:760–770. https://doi.org/10.1016/j.bbamcr.2017.02.011

Kovalevich J, Langford D (2013) Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. Neuronal Cell Culture: Methods And Protocols 9–21. https://doi.org/10.1007/978-1-62703-640-5_2

Lefebvre S, Rglen BL, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M (1995) Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80:155–165. https://doi.org/10.1016/0092-8674(95)90460-3

Li DK, Tisdale S, Lotti F, Pellizzoni L (2014) SMN control of RNP assembly: from post-transcriptional gene regulation to motor neuron disease. Semin Cell Develop Biol Elsevier 22–29

Liang S, Fuhrman S, Somogyi R (1998) Reveal, a general reverse engineering algorithm for inference of genetic network architectures. Biocomputing

Mandelker D, Schmidt RJ, Ankala A, Mcdonald Gibson K, Bowser M, Sharma H, Duffy E, Hegde M, Santani A, Lebo M (2016) Navigating highly homologous genes in a molecular diagnostic setting: a resource for clinical next-generation sequencing. Genet Med 18:1282–1289. https://doi.org/10.1038/gim.2016.58

Marx H, Minogue CE, Jayaraman D, Richards AL, Kwiecien NW, Siahpirani AF, Rajasekar S, Maeda J, Garcia K, Valle-Echevarria D (2016) A proteomic atlas of the legume *Medicago truncatula* and its nitrogen-fixing endosymbiont *Sinorhizobium meliloti*. Nat Biotechnol 34:1198–1205. https://doi.org/10.1038/nbt.3681

Mcandrew P, Parsons D, Simard L, Rochette C, Ray P, Mendell J, Prior T, Burghes A (1997) Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. Amer J Hum Genet 60:1411–1422. https://doi.org/10.1086/515465

Messina S, Sframeli M (2020) New treatments in spinal muscular atrophy: positive results and new challenges. J Clin Med 9:2222. https://doi.org/10.3390/jcm9072222

Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AH, Mcpherson JD (1999) A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum Mol Genet 8:1177–1183. https://doi.org/10.1093/hmg/8.7.1177

Ng S-Y, Soh BS, Rodriguez-Muela N, Hendrickson DG, Price F, Rinn JL, Rubin LL (2015) Genome-wide RNA-Seq of human motor neurons implicates selective ER stress activation in spinal muscular atrophy. Cell Stem Cell 17:569–584. https://doi.org/10.1016/j.stem.2015.08.003

Petti AA, Mcisaac RS, Ho-Shing O, Bussemaker HJ, Botstein D (2012) Combinatorial control of diverse metabolic and physiological functions by transcriptional regulators of the yeast sulfur assimilation pathway. Mol Biol Cell 23:3008–3024. https://doi.org/10.1091/mbc.e12-03-0233

Popescu BO, Ceafalan LC, Ioghen OC (2023) SH-SY5Y Cell Line *In Vitro* Models for Parkinson Disease Research — Old Practice for New Trends. J Integrative Neurosci 22:20. https://doi.org/10.31083/j.jin2201020

Reedich EJ, Kalski M, Armijo N, Cox GA, Didonato CJ (2021) Spinal motor neuron loss occurs through a p53-and-p21-independent mechanism in the Smn2B/– mouse model of spinal muscular atrophy. Experim Neurol 337:113587. https://doi.org/10.1016/j.expneurol.2020.113587

Roy S, Lagree S, Hou Z, Thomson JA, Stewart R, Gasch AP (2013) Integrated module and gene-specific regulatory inference implicates upstream signaling networks. PLOS Computational Biol 9:e1003252. https://doi.org/10.1371/journal.pcbi.1003252

Russman BS (2007) Spinal muscular atrophy: clinical classification and disease heterogeneity. J Child Neurol 22:946–951. https://doi.org/10.1177/0883073807305673

Siahpirani AF, Roy S (2017) A prior-based integrative framework for functional transcriptional regulatory network inference. Nucl Acids Res 45:e21-e21. https://doi.org/10.1093/nar/gkw963

SimonCM, Dai Y, Van Alstyne M, Koutsioumpa C, Pagiazitis JG, Chalif JI, Wang X, Rabinowitz JE, Henderson CE, Pellizzoni L (2017) Converging mechanisms of p53 activation drive motor neuron degeneration in spinal muscular atrophy. Cell Rep 21:3767–3780. https://doi.org/10.1016/j.celrep.2017.12.003

Valbuena A, Vega FM, Blanco S, Lazo PA (2006) p53 downregulates its activating vaccinia-related kinase 1, forming a new autoregulatory loop. Mol Cell Biol 26:4782–4793. https://doi.org/10.1128/MCB.00069-06

Wang H, Qian L, Dougherty E (2010) Inference of gene regulatory networks using S-system: a unified approach. Iet Syst Biol 4:145–156. https://digital-library.theiet.org/content/journals/10.1049/iet-syb.2008.0175

Wang J, Foroutan A, Richardson E, Skinner SA, Reilly J, Kerkhof J, Curry CJ, Tarpey PS, Robertson SP, Maystadt I (2022) Clinical findings and a DNA methylation signature in kindreds with alterations in ZNF711. Europ J Hum Genet 30:420–427. https://doi.org/10.1038/s41431-021-01018-1

Wu D, Tan H, Su W, Cheng D, Wang G, Wang J, Ma DA, Dong GM, Sun P (2022) MZF1 mediates oncogene-induced senescence by promoting the transcription of p16INK4A. Oncogene 41:414–426. https://doi.org/10.1038/s41388-021-02110-y

Received April 18, 2023
Received July 25, 2024
Accepted November 18, 2024