МОЛЕКУЛЯРНАЯ БИОЛОГИЯ, 2022, том 56, № 3, с. 465–467

ГЕНОМИКА. ТРАНСКРИПТОМИКА

УДК 577.21:579.23"315

КАРТИРОВАНИЕ РЕГУЛЯТОРНЫХ ЭЛЕМЕНТОВ В 5'- И 3'-НЕТРАНСЛИРУЕМЫХ ОБЛАСТЯХ мРНК SIGLEC-15 С ПОМОЩЬЮ РЕПОРТЕРНОГО АНАЛИЗА[1]

© 2022 г. L. Wang[a], R. Li[a], X. Lai[a], X. Zhang[a], H. Chen[a, *], W. Zhao[a, **]

[a]*Molecular Cancer Research Center, School of Medicine, Shenzhen Campus of Sun Yat-sen University, Shenzhen, Guangdong, 518107 P.R. China*

**e-mail: chenhm69@mail.sysu.edu.cn*

***e-mail: zhaowx5@mail.sysu.edu.cn*

Поступила в редакцию 20.07.2021 г.
После доработки 21.10.2021 г.
Принята к публикации 31.10.2021 г.

Siglec-15 – иммунный супрессор, активируемый в различных типах злокачественных опухолей, рассматривается как новая потенциальная мишень для иммунотерапии опухолей. Однако неясно, каким образом экспрессия *Siglec-15* контролируется в нормальных и опухолевых клетках. С использованием репортерного анализа оценили влияние 5'- и 3'-UTR мРНК Siglec-15 на экспрессию гена. Обнаружено, что 3'-UTR сильно снижает продукцию репортерного белка, тогда как 5'-UTR проявляет умеренное ингибирующее действие Количественное определение стационарного уровня мРНК выявило хорошее соответствие между количеством белка и представленностью мРНК, содержащей 3'-UTR. Напротив, 5'-UTR слабо влияла на уровень мРНК по сравнению с контролем. Измерение времени полужизни мРНК показало, что 3'-UTR способствует деградации мРНК. Тестирование шести укороченных фрагментов 3'-UTR выявило заметную ингибирующую активность пяти из них в четырех проверенных линиях клеток, а шестой – перекрывающий область 993–1317 – обладал более сильной активностью. Интересно, что область 993–1317 содержит предсказанную структуру стебель–петля из 43 н., проявляющую выраженную ингибирующую активность в четырех линиях клеток. Эти результаты свидетельствуют, что 3'-UTR подавляет экспрессию репортерного гена, вызывая ускоренный распад мРНК, возможно, с использованием нескольких *цис*-регуляторных элементов, а 5'-UTR репрессирует экспрессию гена, ингибируя трансляцию. Таким образом, наши данные дают ключ к пониманию механизмов регуляции экспрессии гена *Siglec-15*.

Ключевые слова: *Siglec-15*, 5'-UTR, 3'-UTR, деградация мРНК, трансляция

DOI: 10.31857/S0026898422030181

СПИСОК ЛИТЕРАТУРЫ

1. Wang J., Sun J., Liu L.N., Flies D.B., Nie X., Toki M., Zhang J., Song C., Zarr M., Zhou X., Han X., Archer K.A., O'Neill T., Herbst R.S., Boto A.N., Sanmamed M.F., Langermann S., Rimm D.L., Chen L. (2019) *Siglec-15* as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat. Med.* **25**, 656–666.
2. Angata T., Tabuchi Y., Nakamura K., Nakamura M. (2007) *Siglec-15*: an immune system Siglec conserved throughout vertebrate evolution. *Glycobiology.* **17**, 838–846.
3. Hiruma Y., Hirai T., Tsuda E. (2011) *Siglec-15*, a member of the sialic acid-binding lectin, is a novel regulator for osteoclast differentiation. *Biochem. Biophys. Res. Commun.* **409**, 424–429.
4. Kang F.B., Chen W., Wang L., Zhang Y.Z. (2020) The diverse functions of *Siglec-15* in bone remodeling and antitumor responses. *Pharmacol. Res.* **155**, 104728.
5. Cao G., Xiao Z., Yin Z. (2019) Normalization cancer immunotherapy: blocking *Siglec-15*! *Signal. Transduct. Target. Ther.* **4**, 10.
6. Kameda Y., Takahata,M., Komatsu M., Mikuni S., Hatakeyama S., Shimizu T., Angata T., Kinjo M., Minami A., Iwasaki N. (2013) *Siglec-15* regulates osteoclast differentiation by modulating RANKL-induced phosphatidylinositol 3-kinase/Akt and Erk pathways in association with signaling Adaptor DAP12. *J. Bone Miner Res.* **28**, 2463–2475.
7. Sato D., Takahata M., Ota M., Fukuda C., Tsuda E., Shimizu T., Okada A., Hiruma Y., Hamano H., Hiratsuka S., Fujita R., Amizuka N., Hasegawa T., Iwasaki N.

[1] Статья представлена авторами на английском языке.

(2018) *Siglec-15*-targeting therapy increases bone mass in rats without impairing skeletal growth. *Bone.* **116**, 172–180.

8. Mayr C. (2019) What Are 3' UTRs Doing? *Cold Spring Harb. Perspect. Biol.* **11**(10), a034728.
9. Zhao W., Pollack J.L., Blagev D.P., Zaitlen N., McManus M.T., Erle D.J. (2014) Massively parallel functional annotation of 3' untranslated regions. *Nat. Biotechnol.* **32**, 387–391.
10. Brennan S.E., Kuwano Y., Alkharouf N., Blackshear P.J., Gorospe M., Wilson G.M. (2009) The mRNA-destabilizing protein tristetraprolin is suppressed in many cancers, altering tumorigenic phenotypes and patient prognosis. *Cancer Res.* **69**, 5168–5176.
11. Zhao W., Erle D.J. (2018) Widespread effects of chemokine 3' untranslated regions on mRNA degradation and protein production in human cells. *J. Immunol.* **201**, 1053–1061.
12. Guo H., Ingolia N.T., Weissman J.S., Bartel D.P. (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature.* **466**, 835–840.
13. An J.J., Gharami K., Liao G.Y., Woo N.H., Lau A.G., Vanevski F., Torre E.R., Jones K.R., Feng Y., Lu B., Xu B. (2008) Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell.* **134**, 175–187.
14. Chen S., Wang R., Zheng D., Zhang H., Chang X., Wang K., Li W., Fan J., Tian B., Cheng H. (2019) The mRNA export receptor NXF1 coordinates transcriptional dynamics, alternative polyadenylation, and mRNA export. *Mol. Cell.* **74**, 118–131. e117.
15. Ma W., Mayr C. (2018) A Membraneless organelle associated with the endoplasmic reticulum enables 3'UTR-mediated protein-protein interactions. *Cell.* **175**, 1492–1506. e1419.
16. Ingolia N.T., Ghaemmaghami S., Newman J.R., Weissman J.S. (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science.* **324**, 218–223.
17. Johnstone T.G., Bazzini A.A., Giraldez A.J. (2016) Upstream ORFs are prevalent translational repressors in vertebrates. *EMBO J.* **35**, 706–723.
18. Hinnebusch A.G., Ivanov I.P., Sonenberg N. (2016) Translational control by 5'-untranslated regions of eukaryotic mRNAs. *Science.* **352**, 1413–1416.
19. Rogers J.T., Randall J.D., Cahill C.M., Eder P.S., Huang X., Gunshin H., Leiter L., McPhee J., Sarang S.S., Utsuki T., Greig N.H., Lahiri D.K., Tanzi R.E., Bush A.I., Giordano T., Gullans S.R. (2002) An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. *J. Biol. Chem.* **277**, 45518–45528.
20. Jia L., Mao Y., Ji Q., Dersh D., Yewdell J.W., Qian S.B. (2020) Decoding mRNA translatability and stability from the 5' UTR. *Nat. Struct. Mol. Biol.* **27**, 814–821.
21. Mayr C. (2017) Regulation by 3'-untranslated regions. *Annu. Rev. Genet.* **51**, 171–194.
22. Leppek K., Schott J., Reitter S., Poetz F., Hammond M.C., Stoecklin G. (2013) Roquin promotes constitutive mRNA decay via a conserved class of stem-loop recognition motifs. *Cell.* **153**, 869–881.
23. Jodoin R., Perreault J.P. (2018) G-quadruplexes formation in the 5'UTRs of mRNAs associated with colorectal cancer pathways. *PLoS One.* **13**, e0208363.
24. Jodoin R., Carrier J.C., Rivard N., Bisaillon M., Perreault J.P. (2019) G-quadruplex located in the 5'UTR of the BAG-1 mRNA affects both its cap-dependent and cap-independent translation through global secondary structure maintenance. *Nucleic Acids Res.* **47**, 10247–10266.
25. Ishida-Kitagawa N., Tanaka K., Bao X., Kimura T., Miura T., Kitaoka Y., Hayashi K., Sato M., Maruoka M., Ogawa T., Miyoshi J., Takeya T. (2012) *Siglec-15* protein regulates formation of functional osteoclasts in concert with DNAX-activating protein of 12 kDa (DAP12). *J. Biol. Chem.* **287**, 17493–17502.
26. Stuible M., Moraitis A., Fortin A., Saragosa S., Kalbakji A., Filion M., Tremblay G.B. (2014) Mechanism and function of monoclonal antibodies targeting *siglec-15* for therapeutic inhibition of osteoclastic bone resorption. *J. Biol. Chem.* **289**, 6498–6512.
27. Sun J., Lu Q., Sanmamed M.F., Wang J. (2021) *Siglec-15* as an emerging target for next-generation cancer immunotherapy. *Clin. Cancer Res.* **27**, 680–688.
28. Andersson R. (2015) Promoter or enhancer, what's the difference? Deconstruction of established distinctions and presentation of a unifying model. *Bioessays.* **37**, 314–323.
29. Kuersten S., Goodwin E.B. (2003) The power of the 3' UTR: translational control and development. *Nat. Rev. Genet.* **4**, 626–637.
30. Chen M., Lyu G., Han M., Nie H., Shen T., Chen W., Niu Y., Song Y., Li X., Li H., Chen X., Wang Z., Xia Z., Li W., Tian X.L., Ding C., Gu J., Zheng Y., Liu X., Hu J., Wei G., Tao W., Ni T. (2018) 3' UTR lengthening as a novel mechanism in regulating cellular senescence. *Genome Res.* **28**(3), 285–294. https://doi.org/10.1101/gr.224451.117
31. Schuster S.L., Hsieh A.C. (2019) The untranslated regions of mRNAs in cancer. *Trends Cancer.* **5**, 245–262.
32. Kataoka K., Shiraishi Y., Takeda Y., Sakata S., Matsumoto M., Nagano S., Maeda T., Nagata Y., Kitanaka A., Mizuno S., Tanaka H., Chiba K., Ito S., Watatani Y., Kakiuchi N., Suzuki H., Yoshizato T., Yoshida K., Sanada M., Itonaga H., Imaizumi Y., Totoki Y., Munakata W., Nakamura H., Hama N., Shide K., Kubuki Y., Hidaka T., Kameda T., Masuda K., Minato N., Kashiwase K., Izutsu K., Takaori-Kondo A., Miyazaki Y., Takahashi S., Shibata T., Kawamoto H., Akatsuka Y., Shimoda K., Takeuchi K., Seya T., Miyano S., Ogawa S. (2016) Aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. *Nature.* **534**, 402–406.
33. Chatterjee S., Pal J.K. (2009) Role of 5'- and 3'-untranslated regions of mRNAs in human diseases. *Biol Cell.* **101**, 251–262.
34. Goodarzi H., Zhang S., Buss C.G., Fish L., Tavazoie S., Tavazoie S.F. (2014) Metastasis-suppressor transcript

destabilization through TARBP2 binding of mRNA hairpins. *Nature.* **513**, 256–260.

35. Turi A., Loglisci C., Salvemini E., Grillo G., Malerba D., D'Elia D. (2009) Computational annotation of UTR cis-regulatory modules through frequent pattern mining. *BMC Bioinformatics.* **10**(Suppl. 6), S25.
36. Zhao W., Siegel D., Biton A., Tonqueze O.L., Zaitlen N., Ahituv N., Erle D.J. (2017) CRISPR-Cas9-mediated functional dissection of 3'-UTRs. *Nucl. Acids Res.* **45**, 10800–10810.
37. Chendrimada T.P., Gregory R.I., Kumaraswamy E., Norman J., Cooch N., Nishikura K., Shiekhattar R. (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature.* **436**, 740–744.
38. Schneider B.D., Leibold E.A. (2003) Effects of iron regulatory protein regulation on iron homeostasis during hypoxia. *Blood.* **102**, 3404–3411.
39. Kulkarni S., Savan R., Qi Y., Gao X., Yuki Y., Bass S.E., Martin M.P., Hunt P., Deeks S. G., Telenti A., Pereyra F., Goldstein D., Wolinsky S., Walker B., Young H.A., Carrington M. (2011) Differential microRNA regulation of HLA-C expression and its association with HIV control. *Nature.* **472**, 495–498.

MAPPING REGULATORY ELEMENTS WITHIN 5' AND 3' UTRs OF *SIGLEC15* WITH A USE OF REPORTER SYSTEM

L. Wang[1], R. Li[1], X. Lai[1], X. Zhang[1], H. Chen[1, *], and W. Zhao[1, **]

[1] Molecular Cancer Research Center, School of Medicine, Shenzhen Campus of Sun Yat-sen University, Shenzhen, Guangdong, 518107 P.R. China

**e-mail: chenhm69@mail.sysu.edu.cn*

***e-mail: zhaowx5@mail.sysu.edu.cn*

Siglec-15 is an immune suppressor with broad upregulation on various cancer types and has emerged as a potential target for cancer immunotherapy. However, it remains unclear how *SIGLEC15* expression is controlled in normal or cancer cells. In this work, we utilized reporter assays to evaluate the impact of the 5' UTR and the 3' UTR of *SIGLEC15* mRNA on gene expression. We found that the 3' UTR dramatically reduced reporter protein production, whereas the 5' UTR showed modest inhibitory effect. Quantification of steady-state mRNA revealed the good coupling of protein amount and mRNA abundance that was associated with the 3' UTR. In contrast, the 5' UTR had little effect on mRNA abundance compared with the empty control. By measuring mRNA half-life, we showed that the 3' UTR markedly promoted mRNA degradation. Testing shortened 3' UTR fragments demonstrated five out of the six having notable inhibitory effect, with the one spanning 993–1317 had the most robust activity. More interestingly, the 993–1317 region contains a predicted 43-nt stem-loop structure that showed apparent inhibitory activity in four cell lines tested. These results suggested that the 3' UTR inhibited reporter gene expression by accelerating mRNA decay possibly via multiple *cis*-regulatory elements, but the 5' UTR repressed gene expression by inhibiting translation. Thus, our findings provided a clue to the molecular mechanism underlying the regulation of *SIGLEC15* expression.

Keywords: *SIGLEC15*, 5' UTR, 3' UTR, mRNA degradation, translation