ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2018; 17(3):274-280.

Expression of TNF- and HNRNPL-related Immunoregulatory Long Non-coding RNA (THRIL) in Acute Myeloid Leukemia: Is There Any Correlation?

Arezou Sayad[1], Abbas Hajifathali[2], Mir Davood Omrani[1,3], Shahram Arsang-Jang[4], Amir Ali Hamidieh[5], and Mohammad Taheri[1,3]

[1] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Taleghani Bone Marrow Transplantation Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Epidemiology and Biostatistics, Faculty of Health, Qom University of Medical Sciences, Qom, Iran
[5] Department of Pediatric Stem Cell Transplantation, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Received: 9 August 2017; Received in revised form: 12 December 2017; Accepted: 4 February 2018

ABSTRACT

Recently, Long noncoding RNAs (lncRNAs) have been described as regulatory factors for several biological mechanisms through regulating the gene expression. Among them the TNF and HNRNPL related immunoregulatory (THRIL) lncRNA may be involved in the pathogenesis of immune-related and inflammatory disease through controlling the expression of the tumor necrosis factor-alpha (TNF-α) expression.

In this case-control study, we investigate the THRIL expression in blood 25 samples of de novo acute myeloid leukemia (AML) cases (10 females and 15 males, mean age±SD: 35.1±3.2 years) in comparison to 50 healthy age and sex matched controls (21 females and 29 males, mean age±SD: 34.9± 3.1) using real-time quantitative reverse transcription-PCR (qRT-PCR) in order to explore any association between THRIL and AML.

Our results revealed that there was no significant difference in the expression level of THRIL lncRNA between AML patients and healthy individuals (p=0.2, 95% CI=-0.129-28.35). In addition, there was no significant association between male subgroup and THRIL expression as well as females (p=0.08, 95% CI=-0.197-19.251, p=0.4, 95% CI=-0.185-12.041, respectively). In comparison between control group and FAB classification subtypes of AML patients, there was not any significant association.

In conclusion, our study showed that THRIL cannot be used as an informative biomarker for AML diagnosis, however, our results need to be clarify by evolution of more cases.

Keyword: Acute myeloid leukemia; Expression analysis; Long non-coding RNA

Corresponding Author: Mohammad Taheri, PhD; Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, 8th Floor, SBUMS Bldg., Evin, Tehran, Iran.
PO.Box: 198396-3113, Tel: (+98 21) 7763 5431, Fax: (+98 21) 7763 5431, E-mail: Mohammad_823@yahoo.com

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Acute myeloid leukemia (AML) is a myelogenous hematological cancer in which aberrant proliferation of immature hematopoietic stem cells (HSCs) leads to malignant accumulation of them in the peripheral blood, bone marrow, and other tissues. AML is the most common leukemia in adults that accounts for 90% of all cases with annual incidence around 3.5 per 100,000 person and it is 10 fold more prevalent in elderly people with ≤65 years.[1]

The heterogeneous etiology of the disease is not completely explored and the environmental factors such as exposure to diagnostic or therapy-related radiations as well as different genetic factors have been reported to be involved in the pathology of the disease.[2] Aberrant expression of different genes involved in the regulatory pathways of HSCs lineage commitment has been reported in AML patients. These defects leads to malignant proliferation and accumulation of immature blood cells in the patients.[3] As genome wide association studies have noted various immune-related genes located in the 6p21.3 regions such as TNFa and HLA with several hematopoietic malignancies, we investigated the association of these region genes with some other malignancies, previously.[4-8] On the other hand, imbalanced expression of the gene regulatory factors such as Long non-coding RNAs (lncRNAs) are considered as a causative factor in different cancers include AML.[9-12] Among them, TNF and HNRNPL related immunoregulatory long non-coding RNA (THRIL) is an immune-related lncRNA that expressed in several tissues and it is required to control the expression of the tumor necrosis factor (TNFα) gene.[13] TNFα that encodes a pro-inflammatory cytokine has regulate different fundamental cell processes such as cell proliferation, differentiation, and apoptosis and has implicated in different human diseases, including autoimmune disorders and various cancer.[14]

Since, several lines of evidence have indicated the role of inflammatory cytokines such as TNFα in the onset and progress of hematological malignancies, the defective expression of this gene as a consequence of the THRIL lncRNA deregulation may be a causative and also a promising diagnostic biomarker for the AML disease. In this regard, in the present study, the expression level of the THRIL lncRNA was assessed in AML patients in comparison to healthy controls in order to define the correlation between the expression of THRIL lncRNA and the disease.

MATERIALS AND MERTHODS

Subjects

The participants in the present case-control study consist of 25 unrelated adult

AML patients include 10 females (40%) and 15 males (60%) and 50 age and sex matched controls (21 females (42%) and 29 males (58%)). The diagnosis of all the AML patients were clinically defined according to the French–American–British (FAB) classification criteria.[15] FAB classification system refers to a series of classifications of hematologic diseases. It is based on the presence of dysmyelopoiesis and the quantification of myeloblasts and erythroblasts. They were collected from Medical Oncology department of Besat Hospital, Hamadan. The control group consists of 50 individuals that were totally healthy without any cancer history or other disease like genetic syndromes or metabolic disorders.

Sample Collection

After explaining the experiment to the individuals the written consent forms were received from all cases and controls and their complete personal and familial history were obtained. Five ml of peripheral blood was obtained from each participant and transferred to an EDTA tube. The study was approved by a local Ethical Committee of Hamedan University of Medical Sciences (N. IR.UMSHA.REC.1395.383).

Real-Time Quantitative RT-PCR

The General Hybrid-RTM blood RNA extraction Kit (cat No. 305-101) was used to extract total RNA from blood samples. Applied Biosystems High-Capacity cDNA Reverse Transcription Kits (PN: 4375575) was conducted to synthesize the single strand cDNA according to the manufacturer's instructions. Allele ID 7 (Premier Biosoft, Palo Alto, USA) was applied to design the specific probes and primers. The expression level of HPRT1 gene was as considered as a housekeeping gene to normalize the gene expression level of each sample. The sequence of probes and primer pairs has been demonstrated in Table 1. Real-time quantitative PCR was carried out in triplicates by using Applied Biosystems TaqMan R Universal PCR Master Mix (PN: 4304449). Corbett Rotor-gene 6000 machine (Corbett Life Science) was used to run the reactions. The negative control sample was used without cDNA sample as quality control.

Table1. The sequences of probes and primers for TNF- and HNRNPL-related Immunoregulatory (THRIL) lncRNA and hypoxanthine phosphoribosyltransferasegene

property	HPRT1	THRIL
Forward primer	AGCCTAAGATGAGAGTTC	GAGTGCAGTGGCGTGATCTC
Reverse primer	CACAGAACTAGAACATTGATA	AAAATTAGTCAGGCATGGTGGTG
Probe	FAM-CATCTGGAGTCCTATTGACATCGC-TAMRA	FAM- CTCACCGCAACCTCCACCTCCCAG-TAMRA

Statistical Analysis

The obtained data was analyzed using the independent samples t-test to examine the differences between two independent groups. Also, the one-way ANOVA test was used to compare means among control and MS groups and Pearson correlation coefficient were used. The Shapiro-Wilks test was used to investigate the normality except for small sample size, in which non-parametric method was used for small sample size. Finally, due to the same estimates of non-parametric method compared to parametric tests the results of parametric tests were reported. The *p* value<0.05 was considered as significant statistically. All of the analyses were performed using SPSS 18 windows statistical package (Chicago, IL, USA).

RESULTS

Clinical and Demographic Information

For all the samples clinical characteristics include complete blood count, age, and age at onset are described in Table 2. The age of patient and control group was 35.1± 3.2 with the age range of 22-55 years and 34.9 ± 3.1 with the age range of 20-58 years, respectively. Based on FAB classification, AML subtypes were 2 (8%) M0, 3 (12%) M1, 6 (24%) M2, 2 (8%) M3, 6 (36%) M4, 3 (12%) M5.

Expression Analysis for THRIL lncRNA in Cases and Controls

The analysis data of THRIL expression are

Table 2. Demographic and clinical data of acute myeloid leukemia patients and healthy controls

Variables	AML patient	Healthy Control
Female/Male (no. (%))	10(40%)/15(60%)	21(42%)/29(58%)
Age (mean ± SD, Y)	35.1± 3.2	34.9± 3.1
Age range (Y)	22-55	20-58
Age of onset (mean ± SD, Y)	34.8± 4.2	-
WBC (mean ± SD, $\times10^3$)	47± 3.3	6± 2.4
WBC range ($\times10^3$)	18-130	4-7
Platelet (mean ± SD, $\times10^3$)	64± 4.9	220± 1.9
Platelet range ($\times10^3$)	40-250	160-400
Hemoglobin (mean ± SD, g/dl)	8± 3.1	14± 2.3
Hemoglobin range ($\times10^3$, g/dl)	4-11	12-18
FAB classification: (no. (%))	25(100%)	
M0	2(8%)	
M1	3(12%)	
M2	6(24%)	
M3	2(8%)	
M4	9(36%)	
M5	3(12%)	

demonstrated in Table 3. Totally, no significant statistical differences were found between patients and healthy controls (*p*-value=0.2, 95% CI=-0.129-28.35). We also analyzed the obtained data in the male and female subgroup to find out any possible association between gender and the expression level of THRIL for susceptibility to AML. There is no significant association between male subgroup and THRIL expression in comparing patients and controls as well as females (*p*-value=0.08, 95% CI=-0.197-19.251, *p*-value=0.4, 95% CI=-0.185-12.041, respectively). Figure 1 demonstrates the actual data points of THRIL expression level in the AML patients, controls and their gender category. In addition, the comparison between control group and FAB classification subtypes of AML patients showed no significant association (*p*-values> 0.05) (Table 3). The actual data points of THRIL expression level in the groups is demonstrated in Figure 2.

Table 3. Expression in comparing of de novo acute myeloid leukemia (AML) patients and healthy controls

THRIL expression	Control no.	AML patient no.	*p* value	Expression ratio	Std. Error	95% CI
Total	50	25	0.2	1.283	0.549	-0.129-28.35
Male	29	15	0.08	1.306	0.681	-0.197-19.251
Female	21	10	0.4	1.198	1.04	-0.185-12.041
FAB Classification						
M0	50	2	0.09	1.401	1.263	-0.328-8.22
M1	50	3	0.1	1.399	0.97	-0.121-9.85
M2	50	6	0.2	1.01	0.674	-0.011-0.898
M3	50	2	0.08	1.115	1.453	-0.194-9.091
M4	50	9	0.3	1.011	1.01	-0.23-10.08
M5	50	3	0.2	1.2	0.872	-0.105-11.957

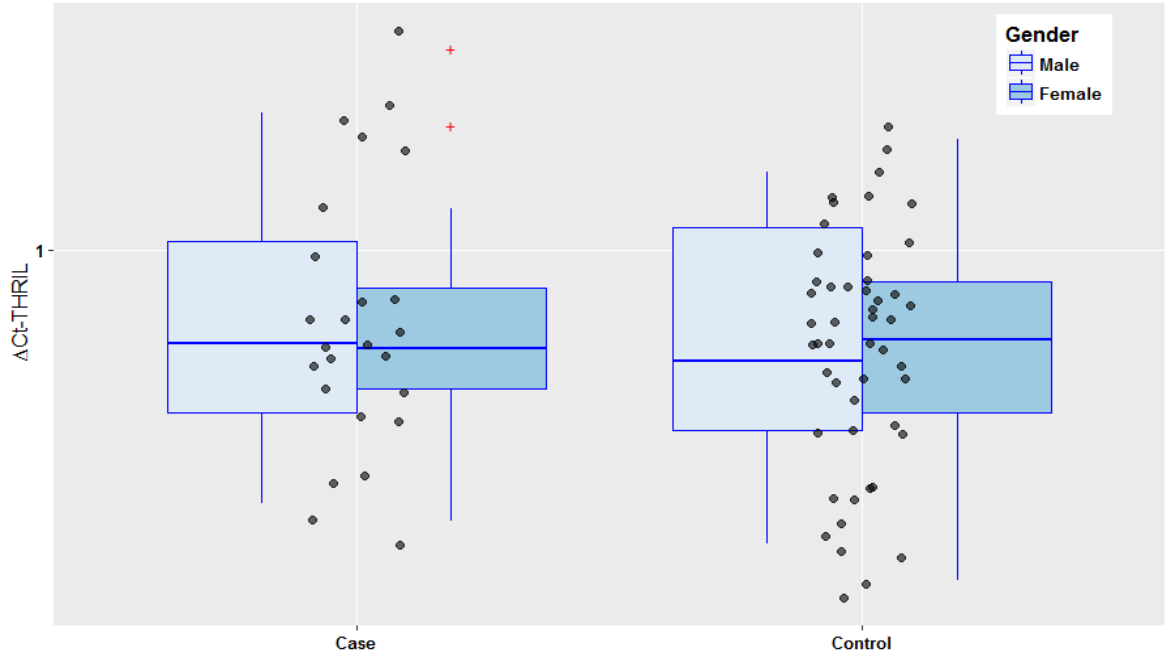

Figure 1. The actual data points of TNF- and HNRNPL-related Immunoregulatory (THRIL) expression level in the acute myeloid leukemia (AML) patients, controls and their gender category. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers, and the outliers are plotted individually using the '+' symbol.

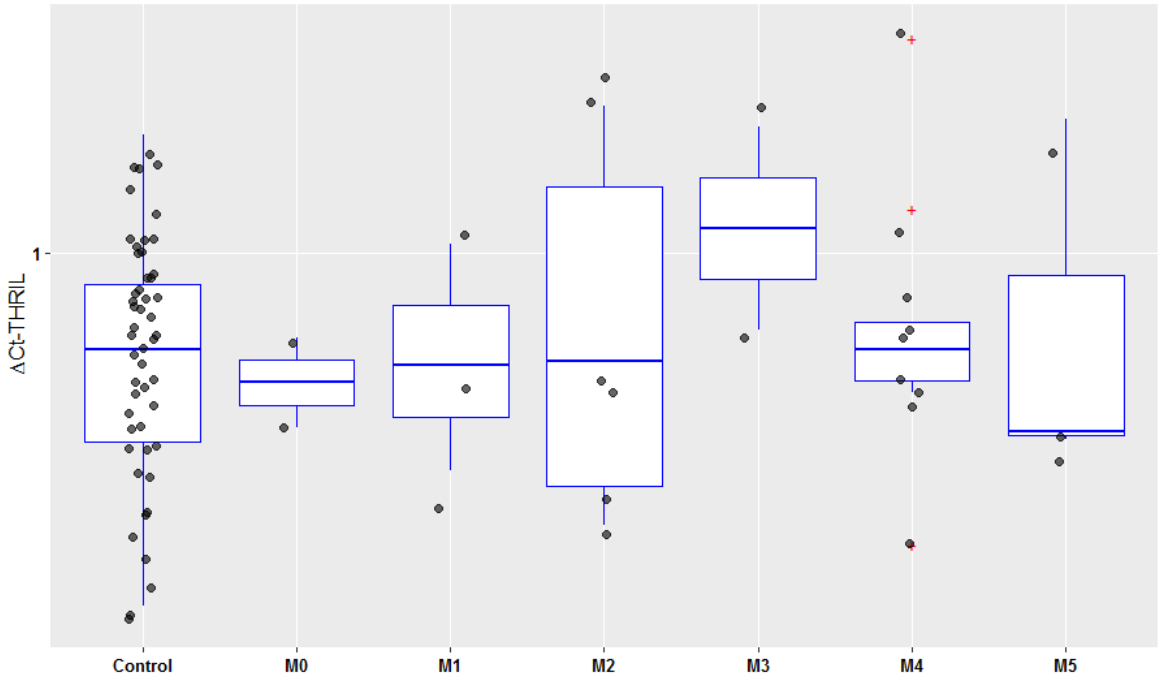

Figure 2. The actual data points of TNF- and HNRNPL-related (THRIL) expression level in the FAB classification subtypes of patients and controls. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers, and the outliers are plotted individually using the '+' symbol.

DISCUSSION

Almost all the Hematological malignancies such as acute myeloid leukemia (AML) are influenced by inflammatory environment and cytokines through their initial steps and progression. Among these inflammatory factors, Tumor necrosis factor-alpha (TNF-α) is a major regulatory cytokine that involved in the pathogenesis of several immune-related hematologic malignancies including AML.[16] The TNF-α affect the three main characteristics of the AML disease that disrupt the normal hematopoiesis consist of aberrant hematopoietic cell differentiation, continuous proliferation of progenitor cells, and accumulation of immature cells in peripheral tissues.[17] Previous studies have shown an elevated level of TNF-α in AML patients than healthy controls.[18-20]

Besides, one of the most recent regulatory factors for the gene expression are Long noncoding RNA (lncRNAs).[21] Previous studies have shown a correlation between deregulated expression of lncRNAs and different human disease such as cancers.[22] They play a critical regulatory role in several biological mechanisms that control gene expression at translational or transcriptional levels.[12,23] In particular, lncRNAs have a pivotal regulatory role in hematopoiesis during different steps of HSCs development.[9-11] Among them the THRIL lncRNA may be involved in the pathogenesis of immune-related and inflammatory disease through controlling the expression of the TNF-α expression.[24] THRIL is transcribed from the opposite strand of the gene encoded the BRI3 binding protein (Bri3bp) located at 12q24.31 downstream of the gene.[24]

It has been shown that THRIL lncRNA has increased the TNF transcription through creating a complex with hnRNPL and binding to the promoter of the gene. It can also interact with heterogeneous RNA-binding proteins and thereby modulate the immune response of human THP1 macrophages downstream of Toll-like receptor signaling. On the other hand, the expression of THRIL in THP1 macrophages could indirectly decreased by the Pam3CSK4, a Toll-like receptor 1/2 (TLR1/2) agonist that stimulates the release of TNF-α in macrophages. Also, it's estimated that THRIL knockdown induced a decreased

expression of several genes and blocked the different expression of many other genes in THP1 macrophages.[25] According to the important role of THRIL lncRNA in immune regulatory mechanisms as well as the pathogenesis of immune related disease we focused on the role off this lncRNA in the AML disease.

The investigation of the expression level of THRIL in the blood sample of AML patients in comparison to healthy controls were done in order to find a potential diagnostic biomarker for early diagnosis of AML or even better understanding of the underlying mechanism of the disease. The results of the study showed that the expression level of THRIL is not significantly different between AML cases and healthy controls.

According to our knowledge, this is the first study to analysis the correlation between THRIL expression and AML. The obtained data indicated that there is no significantly association between the expression of THRIL and the disease in total cases or even in two separated sex groups. The relatively small sample size of the study group is as a limitation of our study. Although, our results indicated that the THRIL could not be considered as a biomarker for AML most investigations are needed to clear the exact relation between THRIL and the AML disease.

Overall, our study indicated that there is no significant difference in the expression level of THRIL lncRNA between AML cases and healthy controls in total or in separated sex groups. However, due to the lack of previous data further analyses are needed to confirm the real correlation between THRIL and AML.

ACKNOWLEDGEMENTS

This study was supported financially by Shahid Beheshti University of Medical Sciences grant number (14120.

REFERENCES

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015; 373(12):1136-52.
2. Leone G, Pagano L, Ben-Yehuda D, Voso MT. Therapy-related leukemia and myelodysplasia: susceptibility and incidence. Haematologica 2007; 92(10):1389-98.
3. Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, van Doorn-Khosrovani SBvW, Boer JM, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 2004; 350(16):1617-28.
4. Taheri M, Nemati S, Movafagh A, Saberi M, Mirfakhraie R, Eftekharian MM, Arsang-Jang S, Rezagholizadeh A, Sayad A. TRAIL gene expression analysis in multiple sclerosis patients. Hum Antibodies 2016; 24(1-2):33-8.
5. Sayad A, Hajifathali A, Hamidieh AA, Roshandel E, Taheri M. HOTAIR Long Noncoding RNA is not a Biomarker for Acute Myeloid Leukemia (AML) in Iranian Patients. Asian Pac J Cancer Prev 2017; 18(6):1581-4.
6. Sayad A, Hajifathali A, Taheri M. Polymorphisms in lncRNA HOTAIR and Susceptibility to Acute Myeloid Leukemia in Iranian Patients. Journal of Biology and Today's World 2017; 6(2):33-7.
7. Sayad A, Akbari MT, Mehdizadeh M, Taheri M, Hajifathali A. HLA-A*26 and Susceptibility of Iranian Patients with Non-Hodgkin Lymphoma. Iran J Immunol 2014; 11(4):269-274.
8. Khorshidi HR, Taheri M, Noroozi R, Soudyab M, Sayad A, Ghafouri-Fard S. Investigation of the association of HOTAIR single nucleotide polymorphisms and risk of breast cancer in an Iranian population. International Journal of Cancer Management 2017;10(5).
9. Taheri M, Omrani MD, Ghafouri-Fard S. Long non-coding RNA expression in bladder cancer. Biophysical reviews 2017.
10. Taheri M, Pouresmaeili F, Omrani MD, Habibi M, Sarrafzadeh S, Noroozi R, Rakhshan A, Sayad A, Ghafouri-Fard S. Association of ANRIL gene polymorphisms with prostate cancer and benign prostatic hyperplasia in an Iranian population. Biomarkers in medicine 2017; 11(5):413-22.
11 Hu W, Yuan B, Flygare J, Lodish HF. Long noncoding RNA-mediated anti-apoptotic activity in murine erythroid terminal differentiation. Genes Dev 2011; 25(24):2573-8.
12. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 2013; 14(11):699-712.
13. Cai B, Wu Z, Liao K, Zhang S. Long noncoding RNA HOTAIR can serve as a common molecular marker for lymph node metastasis: a meta-analysis. Tumour Biol 2014; 35(9):8445-50.
14. Wajant H. The role of TNF in cancer. Death Receptors and Cognate Ligands in Cancer. Springer 2009; 1-15.
15. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. Ann Intern Med 1985; 103(4):620-5.

16. Tsimberidou A-M, Giles FJ. TNF-α targeted therapeutic approaches in patients with hematologic malignancies. Expert Rev Anticancer Ther 2002; 2(3):277-86.
17. Petrides PE, Dittmann KH. How do normal and leukemic white blood cells egress from the bone marrow? Blut 1990; 61(1):3-13.
18. Sanchez-Correa B, Bergua JM, Campos C, Gayoso I, Arcos MJ, Bañas H, et al. Cytokine profiles in acute myeloid leukemia patients at diagnosis: survival is inversely correlated with IL-6 and directly correlated with IL-10 levels. Cytokine 2013; 61(3):885-91.
19. Tsimberidou AM, Estey E, Wen S, Pierce S, Kantarjian H, Albitar M, et al. The prognostic significance of cytokine levels in newly diagnosed acute myeloid leukemia and high-risk myelodysplastic syndromes. Cancer 2008; 113(7):1605-13.
20. Aguayo A, Kantarjian H, Manshouri T, Gidel C, Estey E, Thomas D, et al. Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 2000; 96(6):2240-5.
21. Kornienko AE, Guenzl PM, Barlow DP, Pauler FM. Gene regulation by the act of long non-coding RNA transcription. BMC Biol 2013; 11(1):59.
22. Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015; 21(11):1253-61.
23. Kurokawa R. Long noncoding RNA as a regulator for transcription. Long Non-Coding RNAs. Springer 2011; 29-41.
24. Li Z, Chao T-C, Chang K-Y, Lin N, Patil VS, Shimizu C, et al. The long noncoding RNA THRIL regulates TNFα expression through its interaction with hnRNPL. Proc Natl Acad Sci U S A 2014; 111(3):1002-7.
25. Carpenter S, Aiello D, Atianand MK, Ricci EP, Gandhi P, Hall LL, et al. A long noncoding RNA mediates both activation and repression of immune response genes. science 2013; 341(6147):789-92.