RNA BIOLOGY
2017, VOL. 14, NO. 7, 827–830
https://doi.org/10.1080/15476286.2017.1312228

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POINT OF VIEW

Deregulation of the non-coding genome in leukemia

Susanna Teppo [a], Merja Heinäniemi [b], and Olli Lohi [a]

[a]Tampere Center for Child Health Research, Faculty of Medicine and Life Sciences, University of Tampere and Tampere University Hospital, Tampere, Finland; [b]Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio, Finland

ABSTRACT

Methodological advances that allow deeper characterization of non-coding elements in the genome have started to reveal the full spectrum of deregulation in cancer. We generated an inducible cell model to track transcriptional changes after induction of a well-known leukemia-inducing fusion gene, ETV6-RUNX1. Our data revealed widespread transcriptional alterations outside coding elements in the genome. This adds to the growing list of various alterations in the non-coding genome in cancer and pinpoints their role in diseased cellular state.

ARTICLE HISTORY
Received 7 February 2017
Revised 21 March 2017
Accepted 24 March 2017

KEYWORDS
eRNA; GRO-seq; leukemia; nascent RNA; transcriptional regulation

Approximately 80 % of the genome is transcribed into RNA species in at least some cell type or at some stage of development.[1,2] Non-coding regulatory (non-housekeeping) RNAs are currently defined by their size, genomic location or presumptive function. Enhancer RNAs (eRNA), which have a length span from 0.1 to 10 kb, mainly fall into the category of long non-coding RNAs (lncRNAs) although they are better defined by their transcriptional regulatory function. Larger clusters of enhancers with multiple transcription factor (TF) binding sites and open chromatin marks are termed super-enhancers and they define cell identity.[3,4] Locations of enhancer elements are often deduced from certain histone marks (H3K4me1, H3K27ac), transcription factor binding profiles (p300), or open chromatin states (eg. DNAse- and ATAC-seq). The development of global nascent RNA sequencing techniques, such as global run-on sequencing (GRO-seq),[5] has revealed that transcription of eRNAs is highly correlated with marks such as H3K27ac (for review see ref. 6) and to transcription at nearby gene promoters,[7,8] and is considered the most reliable mark of an active enhancer.[7,9] The functions of eRNAs are yet unclear: they can be passive byproducts of transcription or function actively in recruitment of transcription factors (reviewed in ref. 10), like in the case of Yin-Yang (YY)1.[11]

Misregulation of ncRNAs is common in cancer although recurrent structural variations have been challenging to find. For example, in a study with whole-genome sequencing of 150 tumor/normal pairs of chronic lymphocytic leukemia, only one recurrent non-coding mutation cluster was found at a potential regulatory element.[12] However, this may also reflect the lacking annotations. We recently analyzed whole genome sequencing data from precursor B-cell acute lymphoblastic leukemia (pre-B-ALL) in the context of chromatin architecture and found that the topologically associated domains with the highest number of breakpoints contained unannotated ncRNAs.[13] Functional studies manipulating lncRNA production in leukemia have shown diverse roles in cancer-related pathways.[14-16] In addition, functional studies on enhancers have highlighted their overall role in cancer, as reviewed in ref. 17. In leukemia, somatic mutation of a non-coding element generated a MYB binding site upstream of oncogenic TAL1 locus, and a deletion of the mutated (but not wild type allele) super-enhancer in a T-ALL cell line decreased expression of TAL1 and impaired cell survival.[18] Altered transcription at enhancers may also result from structural or quantitative changes in both enhancer elements and their regulating proteins. Duplication of NOTCH1-driven MYC enhancer was observed in T-ALL and its relevance demonstrated in a mouse knockout model.[19] Moreover, aberrations in chromatin structure and especially in insulator regions induce abnormal gene expression, as exemplified by activation of TAL1 due to a deletion of upstream insulator element.[20] Misregulated transcription during delicate differentiation processes in haematopoietic precursors may also cause cancer by predisposing to secondary mutations. Convergent transcription and RNA polymerase II stalling strongly correlate with structural variation clusters and seem to provide vulnerable regions for RAG and AID mediated double strand breaks in lymphoma and leukemia.[13,21] Although ncRNA expression profiles using microarray or RNA-seq have been published (eg. refs. 22-26), many nascent transcripts have remained unnoticed because of rapid degradation of several ncRNA species. New methods to address this challenge have emerged, such as GRO-seq, PRO-seq or TT-seq that enable monitoring various nascent transcripts and engaged RNA polymerase II in leukemia.[27,28]

CONTACT Susanna Teppo susanna.teppo@uta.fi Tampere Center for Child Health Research, Faculty of Medicine and Life Sciences, University of Tampere, Laakarinkatu 1, Arvo, Tampere 33520, Finland.

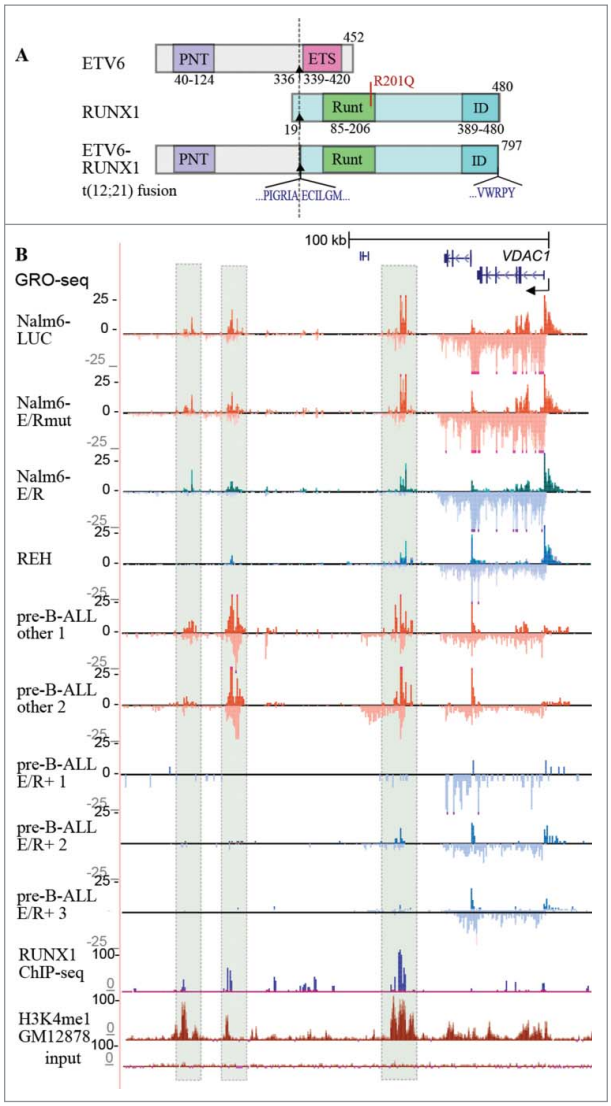

Figure 1. (A) A schematic representation of the ETV6-RUNX1 (E/R, TEL-AML1) fusion protein resulting from a recurrent t(12;21) translocation in pediatric pre-B acute lymphoblastic leukemia. ETV6-RUNX1 includes the pointed (PNT) domain of ETS variant 6 (ETV6) but lacks the ETS domain that is involved in DNA binding of the normal TF protein. The 480 aa long RUNX1 variant 1 (AML-1c, NP_001745) is illustrated with the point mutation R201Q in the Runt domain which impedes its DNA binding capability (this was used to generate E/Rmut in ref. 30). ID = Runx inhibitory domain. (B) GRO-seq signal (nascent RNA transcription) is shown for E/R-negative as red and E/R-positive samples as blue tracks at an example genomic region. Signals above and below the axis indicate plus and minus strands, respectively. RUNX1 ChIP peaks in SEM cells (GSE42075, ref. 42) and an enhancer marker H3K4me1 ChIP-seq in B-cells (GM12878, ref. 2) are shown and coincide with the GRO-seq signal. Three enhancer regions that are downregulated by E/R via RUNX1-mediated binding are highlighted. Nalm6-E/R = 24h expression of E/R in a pre-B-ALL cell line; REH = E/R-positive cell line; pre-B-ALL other = E/R-negative patient; pre-B-ALL E/R+ = E/R-positive patient.

We addressed this issue in the ETV6-RUNX1 (E/R, TEL-AML1) fusion positive leukemia,[30] which represents 25 % of pediatric acute lymphoblastic leukemias, and causes alterations in gene expression that predispose to leukemia.[29] With the help of an inducible E/R cell model and GRO-seq, we explored dynamics of gene expression and the activity of their regulatory elements simultaneously, exposing the transcriptional circuitry downstream of the E/R fusion (Fig. 1).[30] We analyzed enhancers based on eRNA correlation with GRO-seq signal change at differentially expressed genes (transcript-centric approach). Secondly, we generated an enhancer-centric approach that directly applied the statistical framework on eRNA levels to identify significantly regulated enhancers (enhancer annotation was based on H3K27ac and RUNX1 ChIP-seq data) and correlated these changes to that of nearby transcripts. We found at least one similarly altered putative enhancer element within +/−400 kb for almost all the deregulated coding transcripts using transcript-centric approach. E/R regulated approximately 20% of transcribed regions with RUNX1 ChIP peaks, and 5% of CD19/20 (B-cell)-related enhancers. Interestingly, CD19/20 specific super-enhancers were mostly downregulated, implying a way for E/R to arrest cell differentiation.

It has been proposed that any transcription may possess regulatory activity. A recent study showed that half of the studied transcribed gene loci (12 lncRNA and 6 mRNA) regulated a nearby gene in *cis* independently of whether the locus was a coding or non-coding one.[31] As the non-coding genome is only weakly conserved,[1,32] most non-coding regions may function in a way which is not dependent on the sequence of transcript itself but rather the sequence of its promoter or its location in the genome. In the case of E/R leukemia, we classified 57 deregulated novel lncRNAs (over 5 kb long) as either potential eRNAs or lncRNAs based on the GRO-seq signal. One fourth of the novel and 3 of 7 annotated transcripts were concordantly differentially expressed in RNA-seq data with 8 E/R-positive and 9 other subtype pre-B-ALL patients.[30] For example, KCNQ1OT1, which acts in epigenetic regulation,[33-35] was upregulated in our E/R cell model GRO-seq and the patient RNA-seq data. Signal changes at ZEB1 and ZEB1-AS1 serve as an example of a simultaneous downregulation of gene and its promoter-associated RNA, with ZEB also being linked to cancer[36,37] and late B cell differentiation.[38] Functional roles of the novel transcripts in E/R leukemia remains to be explored in future. Nascent RNA profiles of diagnostic patient samples of distinct ALL subtypes will give further insights into the derailed transcriptional network downstream of the oncogenic TF fusions.

Already, thousands of regulatory lncRNA transcripts[39] and hundreds of thousands of enhancer regions have been found. It is now known that ncRNAs are widely specific to a certain cell type and developmental stage. For example, most lncRNAs that are expressed at various stages of mouse B cell development are not expressed in a closely related T-cell lineage.[40] A recent study noted that distal regulatory elements varied across distinct haematopoietic lineages so that they are better discriminators of cell identity than mRNA levels.[41] This was also reflected in our work, where we noticed that sample separation based on quantification of global eRNA transcription was equally good as that based on quantification of transcription at protein coding regions.[30] We can assume that the increasing knowledge of the interplay between various elements of genome and their transcriptional products will significantly contribute to our understanding of the diverse types of leukemia and cancer in near future.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

Susanna Teppo http://orcid.org/0000-0003-2569-8030
Merja Heinäniemi http://orcid.org/0000-0001-6190-3439
Olli Lohi http://orcid.org/0000-0001-9195-0797

References

1. ENCODE Project Consortium TEP, Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007; 447:799-816; PMID:17571346; https://doi.org/10.1038/nature05874
2. ENCODE Project Consortium TEP, Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M. An integrated encyclopedia of DNA elements in the human genome. Nature 2012; 489:57-74; PMID:22955616; https://doi.org/10.1038/nature11247
3. Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 2013; 153:307-19; PMID:23582322; https://doi.org/10.1016/j.cell.2013.03.035
4. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-André V, Sigova AA, Hoke HA, Young RA. Super-enhancers in the control of cell identity and disease. Cell 2013; 155:934-47; PMID:24119843; https://doi.org/10.1016/j.cell.2013.09.053
5. Core LJ, Waterfall JJ, Lis JT. Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science 2008; 322:1845-8; PMID:19056941; https://doi.org/10.1126/science.1162228
6. Lam MTY, Li W, Rosenfeld MG, Glass CK. Enhancer RNAs and regulated transcriptional programs. Trends Biochem Sci 2014; 39:170-82; PMID:24674738; https://doi.org/10.1016/j.tibs.2014.02.007
7. Kaikkonen MU, Spann NJ, Heinz S, Romanoski CE, Allison KA, Stender JD, Chun HB, Tough DF, Prinjha RK, Benner C, et al. Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol Cell 2013; 51:310-25; PMID:23932714; https://doi.org/10.1016/j.molcel.2013.07.010
8. Kim T-K, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, et al. Widespread transcription at neuronal activity-regulated enhancers. Nature 2010; 465:182-7; PMID:20393465; https://doi.org/10.1038/nature09033
9. Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, et al. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature 2011; 474:390-4; PMID:21572438; https://doi.org/10.1038/nature10006
10. Takemata N, Ohta K. Role of non-coding RNA transcription around gene regulatory elements in transcription factor recruitment. RNA Biol 2017; 14:1-5; PMID:27763805; https://doi.org/10.1080/15476286.2016.1248020
11. Sigova AA, Abraham BJ, Ji X, Molinie B, Hannett NM, Guo YE, Jangi M, Giallourakis CC, Sharp PA, Young RA. Transcription factor trapping by RNA in gene regulatory elements. Science (80−) 2015; 350:978-81; PMID:26516199; https://doi.org/10.1126/science.aad3346
12. Puente XS, Beà S, Valdés-Mas R, Villamor N, Gutiérrez-Abril J, Martín-Subero JI, Munar M, Rubio-Pérez C, Jares P, Aymerich M, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature 2015; 526:519-24; PMID:26200345; https://doi.org/10.1038/nature14666
13. Heinäniemi M, Vuorenmaa T, Teppo S, Kaikkonen MU, Bouvy-Liivrand M, Mehtonen J, Niskanen H, Zachariadis V, Laukkanen S, Liuksiala T, et al. Transcription-coupled genetic instability marks acute lymphoblastic leukemia structural variation hotspots. Elife 2016; 5:e12068; PMID:26896675; https://doi.org/10.7554/eLife.13087
14. Blume CJ, Hotz-Wagenblatt A, Hüllein J, Sellner L, Jethwa A, Stolz T, Slabicki M, Lee K, Sharathchandra A, Benner A, et al. p53-dependent non-coding RNA networks in chronic lymphocytic leukemia. Leukemia 2015; 29:2015-23; PMID:25971364; https://doi.org/10.1038/leu.2015.119
15. Guo G, Kang Q, Zhu X, Chen Q, Wang X, Chen Y, Ouyang J, Zhang L, Tan H, Chen R, et al. A long noncoding RNA critically regulates Bcr-Abl-mediated cellular transformation by acting as a competitive endogenous RNA. Oncogene 2015; 34:1768-79; PMID:24837367; https://doi.org/10.1038/onc.2014.131
16. Lu Y, Li Y, Chai X, Kang Q, Zhao P, Xiong J, Wang J. Long noncoding RNA HULC promotes cell proliferation by regulating PI3K/AKT signaling pathway in chronic myeloid leukemia. Gene 2017; 607:41-6; PMID:28069548; https://doi.org/10.1016/j.gene.2017.01.004
17. Sur I, Taipale J. The role of enhancers in cancer. Nat Rev Cancer 2016; 16:483-93; PMID:27364481; https://doi.org/10.1038/nrc.2016.62
18. Mansour MR, Abraham BJ, Anders L, Berezovskaya A, Gutierrez A, Durbin AD, Etchin J, Lawton L, Sallan SE, Silverman LB, et al. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. Science (80-) 2014; 346:1373-7; PMID:25394790; https://doi.org/10.1126/science.1259037
19. Herranz D, Ambesi-Impiombato A, Palomero T, Schnell SA, Belver L, Wendorff AA, Xu L, Castillo-Martin M, Llobet-Navás D, Cordon-Cardo C, et al. A NOTCH1-driven MYC enhancer promotes T cell development, transformation and acute lymphoblastic leukemia. Nat Med 2014; 20:1130-7; PMID:25194570; https://doi.org/10.1038/nm.3665
20. Hnisz D, Weintraub AS, Day DS, Valton A-L, Bak RO, Li CH, Goldmann J, Lajoie BR, Fan ZP, Sigova AA, et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. Science (80-) 2016; 351:1454-8; PMID:26940867; https://doi.org/10.1126/science.aad9024
21. Meng F-L, Du Z, Federation A, Hu J, Wang Q, Kieffer-Kwon K-R, Meyers RM, Amor C, Wasserman CR, Neuberg D, et al. Convergent transcription at intragenic super-enhancers targets AID-initiated genomic instability. Cell 2014; 159:1538-48; PMID:25483776; https://doi.org/10.1016/j.cell.2014.11.014
22. Fernando TR, Rodriguez-Malave NI, Waters EV, Yan W, Casero D, Basso G, Pigazzi M, Rao DS. LncRNA expression discriminates karyotype and predicts survival in B-lymphoblastic leukemia. Mol Cancer Res 2015; 13:839-51; PMID:25681502; https://doi.org/10.1158/1541-7786.MCR-15-0006-T
23. Nordlund J, Kiialainen A, Karlberg O, Berglund EC, Göransson-Kultima H, Sønderkær M, Nielsen KL, Gustafsson MG, Behrendtz M, Forestier E, et al. Digital gene expression profiling of primary acute lymphoblastic leukemia cells. Leukemia 2012; 26:1218-27; PMID:22173241; https://doi.org/10.1038/leu.2011.358
24. Ghazavi F, De Moerloose B, Van Loocke W, Wallaert A, Helsmoortel HH, Ferster A, Bakkus M, Plat G, Delabesse E, Uyttebroeck A, et al. Unique long non-coding RNA expression signature in ETV6/RUNX1-driven B-cell precursor acute lymphoblastic leukemia. Oncotarget 2016; 7:73769-80; PMID:27650541; https://doi.org/10.18632/oncotarget.12063
25. Teittinen KJ, Laiho A, Uusimäki A, Pursiheimo J-P, Gyenesei A, Lohi O. Expression of small nucleolar RNAs in leukemic cells. Cell Oncol 2013; 36:55-63; PMID:23229394; https://doi.org/10.1007/s13402-012-0113-5
26. Ronchetti D, Manzoni M, Agnelli L, Vinci C, Fabris S, Cutrona G, Matis S, Colombo M, Galletti S, Taiana E, et al. lncRNA profiling in early-stage chronic lymphocytic leukemia identifies transcriptional fingerprints with relevance in clinical outcome. Blood Cancer J 2016; 6:e468; PMID:27611921; https://doi.org/10.1038/bcj.2016.77
27. Zhao Y, Liu Q, Acharya P, Stengel KR, Sheng Q, Zhou X, Kwak H, Fischer MA, Bradner JE, Strickland SA, et al. High-resolution mapping of RNA polymerases identifies mechanisms of sensitivity and

resistance to BET inhibitors in t(8;21) AML. Cell Rep 2016; 16:2003-16; PMID:27498870; https://doi.org/10.1016/j.celrep.2016.07.032
28. Schwalb B, Michel M, Zacher B, Frühauf K, Demel C, Tresch A, Gagneur J, Cramer P. TT-seq maps the human transient transcriptome. Science (80-) 2016; 352:1225-8; PMID:27257258; https://doi.org/10.1126/science.aad9841
29. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet 2013; 381:1943-55; PMID:23523389; https://doi.org/10.1016/S0140-6736(12)62187-4
30. Teppo S, Laukkanen S, Liuksiala T, Nordlund J, Oittinen M, Teittinen K, Grönroos T, St-Onge P, Sinnett D, Syvänen A-C, et al. Genome-wide repression of eRNA and target gene loci by the ETV6-RUNX1 fusion in acute leukemia. Genome Res 2016; 26:1468-77; PMID:27620872; https://doi.org/10.1101/gr.193649.115
31. Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttman M, Lander ES. Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016; 539:452-5; PMID:27783602; https://doi.org/10.1038/nature20149
32. Ponjavic J, Ponting CP, Lunter G. Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. Genome Res 2007; 17:556-65; PMID:17387145; https://doi.org/10.1101/gr.6036807
33. Sunamura N, Ohira T, Kataoka M, Inaoka D, Tanabe H, Nakayama Y, Oshimura M, Kugoh H. Regulation of functional KCNQ1OT1 lncRNA by β-catenin. Sci Rep 2016; 6:20690; PMID:26868975; https://doi.org/10.1038/srep20690
34. Ribarska T, Goering W, Droop J, Bastian K-M, Ingenwerth M, Schulz WA. Deregulation of an imprinted gene network in prostate cancer. Epigenetics 2014; 9:704-17; PMID:24513574; https://doi.org/10.4161/epi.28006
35. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-DiNardo D, Kanduri C. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol Cell 2008; 32:232-46; PMID:18951091; https://doi.org/10.1016/j.molcel.2008.08.022
36. Lehmann W, Mossmann D, Kleemann J, Mock K, Meisinger C, Brummer T, Herr R, Brabletz S, Stemmler MP, Brabletz T. ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types. Nat Commun 2016; 7:10498; PMID:26876920; https://doi.org/10.1038/ncomms10498
37. Zhang P, Sun Y, Ma L. ZEB1: At the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. Cell Cycle 2015; 14:481-7; PMID:25607528; https://doi.org/10.1080/15384101.2015.1006048
38. Malpeli G, Barbi S, Zupo S, Tosadori G, Scardoni G, Bertolaso A, Sartoris S, Ugel S, Vicentini C, Fassan M, et al. Identification of microRNAs implicated in the late differentiation stages of normal B cells suggests a central role for miRNA targets ZEB1 and TP53. Oncotarget 2017; 8:11809–26; PMID:28107180; https://doi.org/10.18632/oncotarget.14683
39. Hon C-C, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJL, Gough J, Denisenko E, Schmeier S, Poulsen TM, Severin J, et al. An atlas of human long non-coding RNAs with accurate 5′ ends. Nature 2017; 543:199-204; PMID:28241135; https://doi.org/10.1038/nature21374
40. Brazão TF, Johnson JS, Müller J, Heger A, Ponting CP, Tybulewicz VLJ. Long noncoding RNAs in B-cell development and activation. Blood 2016; 128:e10-9; PMID:27381906; https://doi.org/10.1182/blood-2015-11-680843
41. Corces MR, Buenrostro JD, Wu B, Greenside PG, Chan SM, Koenig JL, Snyder MP, Pritchard JK, Kundaje A, Greenleaf WJ, et al. Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. Nat Genet 2016; 48:1193-203; PMID:27526324; https://doi.org/10.1038/ng.3646
42. Wilkinson AC, Ballabio E, Geng H, North P, Tapia M, Kerry J, Biswas D, Roeder RG, Allis CD, Melnick A, et al. RUNX1 is a key target in t(4;11) leukemias that contributes to gene activation through an AF4-MLL complex interaction. Cell Rep 2013; 3(1):116-27; PMID:23352661; https://doi.org/10.1016/j.celrep.2012.12.016