Experimental Hematology

Experimental Hematology 2016;44:913–916

PERSPECTIVE

Transposable elements: The enemies within

Irene Scarfò[a], Elisa Pellegrino[a], Elisabetta Mereu[a], Giorgio Inghirami[a,b], and Roberto Piva[a]

[a]*Department of Molecular Biotechnology and Health Sciences; Center for Experimental Research and Medical Studies, University of Torino, Torino, Italy;* [b]*Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, USA*

(Received 6 May 2016; revised 21 June 2016; accepted 22 June 2016)

Understanding transformation mechanisms other than genetic aberrations has recently captured the attention of cancer researchers. To date, the role of transposable elements (TEs) in tumor development remains largely undefined. However, an increasing number of studies have reported that loss of epigenetic control causes TE reactivation and consequent oncogenic transcription. Here, we discuss principal examples of TEs-driven oncogenesis. Available data suggest that long terminal repeats and long interspersed nuclear elements play a pivotal role as alternative promoters. These findings provide definitive experimental evidence that repetitive elements are a powerful underestimated force toward oncogenesis and open the possibility to new therapeutic treatments.

Nearly two-thirds of the human genome is composed of repetitive sequences derived from transposable elements (TEs) [1]. These elements are DNA sequences able to mobilize within the genome of a host organism and represent a dynamic route toward evolution [2]. TEs are categorized as retrotransposons (Class I) or DNA transposons (Class II). Retrotransposons are further subdivided into long terminal repeats (LTRs), which account for 8% of the human genome, and non-LTRs, which include the long and short interspersed nuclear elements (LINEs and SINEs, respectively). LTRs derive from integration into the germline of ancient infectious retroviruses that have lost the ability to move from one cell to another. Conversely, non-LTR elements retain the functions needed for retrotransposition through autonomous (LINEs) or nonautonomous (SINEs) mechanisms. Therefore, many somatic LINE insertions have been described in different cancer types [3–7]. Several lines of evidence indicate that TEs have contributed significantly to the human genome either by acting as a source of novel genetic loci or by regulating endogenous gene expression [8–10]. TEs that accumulated in host genomes during evolutionary history have been co-opted for new functions in what is considered a form of domestication [11,12]. There are two means of co-opting TEs into genes: a TE may form a new gene entity or can insert within a preexisting gene to produce a new chimeric protein. Moreover, TE insertion upstream of a gene could perturb gene expression by generating alternative promoters or transcription-factor-binding sites. Conversely, insertion within introns could interfere with transcription, alter normal splicing, or polyadenylation patterns [13].

Because repetitive DNA elements can hamper the physiologic gene regulation, their activity is controlled epigenetically during embryonic development by DNA methylation of 5′-C-phosphate-G-3′ dinucleotides [14–17]. It is known that human tumors recurrently display epigenetic silencing of tumor suppressor genes associated with a global decrease of DNA methylation [18]. The perturbation of DNA methylation patterns in cancer cells creates a condition in which repetitive sequences could become transcriptionally active. Several studies have demonstrated that the activity of TE elements fluctuated during tumor evolution and correlated with promoter hypomethylation [19,20]. This phenomenon could result in the activation of protooncogenes or in aberrant transcription that can contribute to transformation or tumor progression.

A growing number of studies have reported the involvement of TEs in promoting human cancers. In particular, TEs have been proven to play a pivotal role as promoters. A first example of alternative transcription driven by the

Offprint requests to: Roberto Piva, Department of Molecular Biotechnology and Health Sciences, University of Turin, via Nizza 52, Turin 10126, TO, Italy; E-mail: roberto.piva@unito.it

0301-472X/Copyright © 2016 ISEH - International Society for Experimental Hematology. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.exphem.2016.06.251

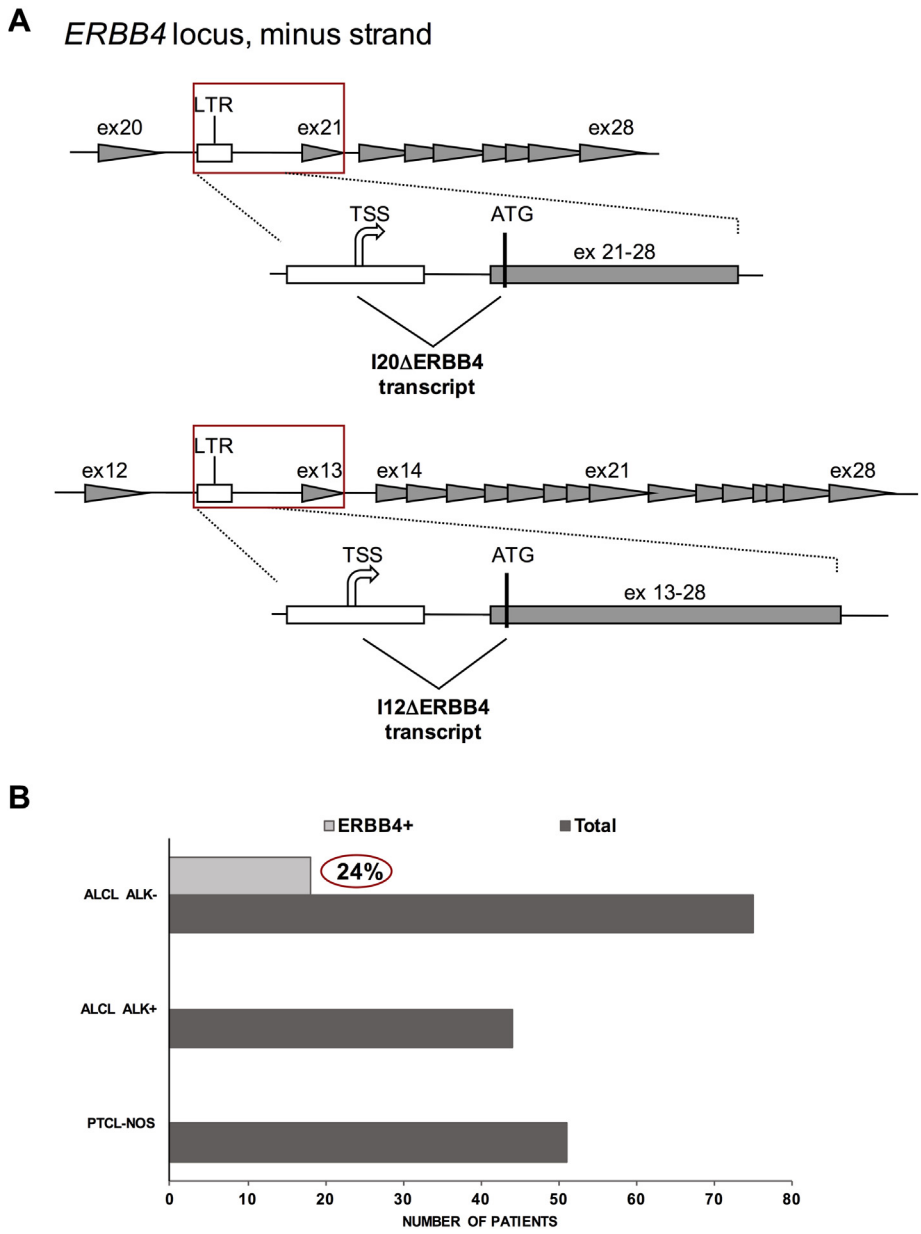

Figure 1. Expression of ERBB4-aberrant transcripts from intronic LTRs. (**A**) Architecture of the human *ERBB4* gene (exons 12–28; *grey* arrowheads). LTRs located in intron 20 and 12 (*white boxes*) are shown. Regions depicting the two aberrant ERRB4s are enlarged. LTR transcription start sites (TSS) are shown by a large arrow. (**B**) Quantitative reverse transcriptase polymerase chain reaction analysis revealed specific expression of I20ΔERBB4 in ∼25% of ALK-negative ALCL samples. A total of 170 samples were analyzed: 51 peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), 44 ALK-positive ALCL, and 75 ALK-negative ALCL.

activation of repetitive elements was described in bladder cancer. Wolff et al. reported that the hypomethylation of a specific LINE-1 promoter induced an alternative transcript of the *MET* oncogene and that the expression of truncated MET protein might affect its signaling properties [21]. However, this study was focused on a single type of TE and a significant role in human malignancies was not demonstrated. Recently, Wiesner et al. identified a novel isoform of the anaplastic lymphoma kinase (*ALK*) gene specifically expressed in ∼11% of melanomas [22]. This aberrant ALK transcript initiates from a de novo alternative transcription site (ATI) containing one LTR and one LINE element. The investigators demonstrated that proteins codified by ATI–ALK can promote tumorigenesis and that ALK–ATI-expressing melanoma cells are sensitive to ALK inhibitors. A more comprehensive investigation in diffuse large B-cell lymphoma (DLBCL) patients was published by Lock et al. [23]. To detect chimeric transcript expression driven by repetitive elements, the investigators conducted a transcriptome-wide analysis in a large cohort of DLBCL patients. The study identified ectopic expression of multiple genes (∼97

chimera transcripts) underlying the impact of TEs on the DLBCL transcriptome. Among these, the LTR2-FABP7 transcript encodes a novel chimeric isoform of the fatty-acid-binding protein 7 (normally expressed in the brain) with altered functions involved in promoting DLBCL cell proliferation.

Significantly, two prominent examples of the oncogenic effect of ancient LTR promoters awakening have been reported in Hodgkin lymphoma (HL). This lymphoma is characterized by the presence of Reed–Sternberg cells, predominantly of B-cell origin, which lose the mature B-cell expression signature. In the first study, Lamprecht et al. demonstrated that the transcriptional activation of a normally dormant endogenous LTR leads to the ectopic expression of the colony-stimulating factor 1 receptor (CSF1R) [24]. The activation of an alternative *CSF1R* promoter is oncogenic and correlates with a poor outcome in HL patients. More recently, Babaian et al. reported the activation of the LOR1a LTR with consequent ectopic overexpression of *IRF5* [25]. Despite IRF5 having been described previously as a key regulator factor with tumor-promoting function in HL [26], the oncogenic role of the LOR1a–IRF5 transcript is still to be demonstrated.

Our group has recently reported an additional example of aberrant LTR-driven oncogenic transcription in ALK-negative anaplastic large cell lymphoma (ALCL) [27]. We have shown that two normally dormant LTRs are located within *ERBB4* introns and can be repurposed, resulting in LTR promoter activation and transcription of two aberrant ERBB4 forms (I20Δ and I12Δ) in ∼25% of ALK-negative ALCL patients (Fig. 1). Our data demonstrated that ERBB4-truncated proteins are required for ALCL growth, even though a role of additional factors in sustaining the tumor phenotype cannot be excluded. However, the epigenetic mechanism responsible for ERBB4-aberrant transcription needs to be defined further. Interestingly, ERBB4-positive cases frequently displayed HL-like features, which are usually rare among ALCL patients. Considering the shared awakening of ancient LTR promoters in HL- and ERBB4-positive ALCL, it will be interesting to analyze *ERBB4*-aberrant expression in HL samples. The identification of a new subclass of ALK-negative ALCL characterized by ectopic expression of aberrant ERBB4 transcripts is important for stratifying peripheral T-cell lymphomas. Furthermore, these findings provide new therapeutic options for innovative target therapies.

Taken together, these data suggest that LTR de-repression and consequent cancer-specific transcriptional activation is a distinct mechanism. Babaian et al. named this process "onco-exaptation," underlining its unique etiology [25]. Such a mechanism for oncogene activation needs to be investigated further to provide new insights into oncogenesis and alternative opportunities for therapeutic interventions. LTR de-repression raises the possibility of the clinical use of epigenetic drugs in TE-driven tumors refractory to current standard therapeutic regimens. Histone methylation and acetylation are among the most relevant modifications that guide chromatin remodeling and epigenetic control [28]. Targeting genes that regulate these modifications can represent valid therapeutic strategies to repress TE-driven oncogenic transcription. In fact, the use of demethylases (e.g., KDM) [29] and bromodomains (e.g., BET) inhibitors to block aberrant transcription could be an attractive option. In particular, numerous studies have highlighted BET inhibitors as a novel category of anticancer agents, with preclinical and clinical evidence in both solid and hematological malignancies [30,31].

Acknowledgments

This work was supported by the Associazione Italiana per la Ricerca sul Cancro, Milan, Italy (AIRC Investigator Grant IG-13358 and Special Program Molecular Clinical Oncology 5 × 1000 No. 10007); Compagnia di San Paolo, Turin, Italy (Grant TO_Call2_2012_0061); and Fondazione CRT, Turin, Italy (Grant 2014_1105).

References

1. Criscione SW, Zhang Y, Thompson W, Sedivy JM, Neretti N. Transcriptional landscape of repetitive elements in normal and cancer human cells. BMC Genomics. 2014;15:583.
2. Brosius J. Retroposons–seeds of evolution. Science. 1991;251:753.
3. Morse B, Rotherg PG, South VJ, Spandorfer JM, Astrin SM. Insertional mutagenesis of the myc locus by a LINE-1 sequence in a human breast carcinoma. Nature. 1988;333:87–90.
4. Miki Y, Nishisho I, Horii A, et al. Disruption of the APC gene by a retrotransposal insertion of L1 sequence in a colon cancer. Cancer Res. 1992;52:643–645.
5. Solyom S, Ewing AD, Rahrmann EP, et al. Extensive somatic L1 retrotransposition in colorectal tumors. Genome Res. 2012;22:2328–2338.
6. Lee E, Iskow R, Yang L, et al. Cancer Genome Atlas Research Network. Landscape of somatic retrotransposition in human cancers. Science. 2012;337:967–971.
7. Villanueva MT. The interloper. Nat Rev Genet. 2015;16:564–565.
8. Mita P, Boeke JD. How retrotransposon shape genome regulation. Curr Opin Genet Dev. 2016;37:90–100.
9. Elbarbary RA, Lucas BA, Maquat LE. Retrotransposons as regulators of gene expression. Science. 2016;351:6274.
10. Thompson PJ, Macfarlan TS, Lorincz MC. Long terminal repeats: from parasitic elements to building blocks of the transcriptional regulatory repertoire. Mol Cell. 2016;62:766–776.
11. Brosius J, Gould SJ. On "genomenclature": a comprehensive (and respectful) taxonomy for pseudogenes and other "junk DNA". Proc Natl Acad Sci U S A. 1992;89:10706–10710.
12. Brosius J. Genomes were forged by massive bombardments with retroelements and retrosequences. Genetica. 1999;107:209–338.
13. Feschotte C. Transposable elements and the evolution of regulatory networks. Nat Rev Genet. 2008;9:397–405.
14. Maksakova IA, Mager DL, Reiss D. Keeping active endogenous retroviral-like elements in check: the epigenetic perspective. Cell Mol Life Sci. 2008;65:3329–3347.
15. Rowe HM, Trono D. Dynamic control of endogenous retroviruses during development. Virology. 2011;411:273–287.
16. Castro-Diaz N, Friedli M, Trono D. Drawing a fine line on endogenous retroelement activity. Mob Genet Elements. 2015;5:1–6.

17. Wolf G, Greenberg D, Macfarlan TS. Spotting the enemy within: targeted silencing of foreign DNA in mammalian genomes by the Krüppel-associated box zinc finger protein family. Mob DNA. 2015;6:17.
18. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics. 2009; 1:239–259.
19. Rodić N, Burns KH. Long interspersed element-1 (LINE-1): passenger or driver in human neoplasms? PLoS One. 2013;9:e1003402.
20. Tubio JM, Li Y, Ju YS, et al. Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes. Science. 2014;345:6196.
21. Wolff EM, Byun HM, Han HF, et al. Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer. PLoS Genet. 2010;6:e1000917.
22. Wiesner T, Lee W, Obenauf AC, et al. Alternative transcription initiation leads to expression of a novel ALK isoform in cancer. Nature. 2015;526:453–457.
23. Lock FE, Rebollo R, Miceli-Royer K, et al. Distinct isoform of FABP7 revealed by screening for retroelement-activated genes in diffuse large B-cell lymphoma. Proc Natl Acad Sci U S A. 2014;111:E3534–E3543.
24. Lamprecht B, Walter K, Kreher S, et al. Derepression of an endogenous long terminal repeat activates the CSF1R proto-oncogene in human lymphoma. Nat Med. 2010;16:571–579.
25. Babaian A, Romanish MT, Gagnier L, et al. Onco-exaptation of an endogenous retroviral LTR drives IRF5 expression in Hodgkin lymphoma. Oncogene. 2016;35:2542–2546.
26. Kreher S, Bouhlel MA, Cauchy P, et al. Mapping of transcription factor motifs in active chromatin identifies IRF5 as key regulator in classical Hodgkin lymphoma. Proc Natl Acad Sci U S A. 2014;111:E4513–E4522.
27. Scarfò I, Pellegrino E, Mereu E, et al. European T-Cell Lymphoma Study Group. Identification of a new subclass of ALK-negative ALCL expressing aberrant levels of ERBB4 transcripts. Blood. 2016;127:221–232.
28. Brien GL, Valerio DG, Armstrong SA. Exploiting the epigenome to control cancer-promoting gene-expression programs. Cancer Cell. 2016;29:464–476.
29. Maes T, Mascaró C, Ortega A, et al. KDM1 histone lysine demethylases as targets for treatments of oncological and neurodegenerative disease. Epigenomics. 2015;7:609–626.
30. Chaidos A, Caputo V, Karadimitris A. Inhibition of bromodomain and extra-terminal proteins (BET) as a potential therapeutic approach in haematological malignancies: emerging preclinical and clinical evidence. Ther Adv Hematol. 2015;6:128–141.
31. Jung M, Gelato KA, Fernández-Montalván A, Siegel S, Haendler B. Targeting BET bromodomains for cancer treatment. Epigenomics. 2015;7:487–501.