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Comment on Tan et al, page 239

Finding ARIEL under the sea of T-ALL circuits

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In this issue of *Blood*, Tan et al report that *ARIEL* (ARID5B-inducing enhancer-associated long noncoding RNA) is a novel enhancer RNA (eRNA) that amplifies oncogenic transcriptional loops in a subset of T-cell acute lymphoblastic leukemia (T-ALL).[1]

As students, many of us learned from the central dogma of molecular biology that information flows from DNA to RNA to protein. In the modern era, genome-scale sequencing has revealed that more than 70% of the genome is transcribed into RNAs that do not produce protein. These RNAs are called noncoding RNAs (ncRNAs). eRNAs have many other names and ambiguous definitions but can be considered simply as ncRNAs that are transcribed from enhancer elements.[2] eRNAs were initially identified through transcriptome sequencing studies, which estimate their numbers in the ~40 000 to 65 000 range. However, conventional sequencing underestimates the number of eRNAs because they are rapidly degraded by the RNA exosome. In contrast, specialized methods such as BruUV-seq in which UV light suppresses RNA exosome activity are more sensitive in detecting eRNAs.[3] Many eRNAs lack function, but others can activate neighboring genes through cis-mediated mechanisms to regulate diverse cellular processes.

In 2014, eRNAs captured the attention of researchers in the field of T-ALL in a report that described the first comprehensive list of long ncRNAs and the first functional eRNA (*LUNAR1*) in human T-ALL.[4] Subsequent studies confirmed the large-scale existence of eRNAs in T-ALL.[5,6] But after 2014, no functionally important eRNAs were identified. Doubts grew. Perhaps *LUNAR1* was a one-hit wonder. Perhaps eRNAs were simply transcriptional byproducts of enhancer activation. These doubts are now gone. Using a stunning variety of sophisticated techniques, Tan et al identified the eRNA *XLOC_005968*, which they named "*ARIEL*," as a new major player in the TAL1-activated subset of T-ALL.

Tan et al previously reported that the transcription factor ARID5B was an important downstream target of the oncogenic TAL1 complex.[7] TAL1 is aberrantly overexpressed in more than 40% of human patients with T-ALL. The TAL1 complex consists of several transcriptional regulators that co-occupy enhancers with partner transcription factors to activate positive feed-forward loops that induce oncogenes such as *MYB* and *MYC*.[7,8] We learned that the TAL1 complex was the key that turned the circuit on. However, there were missing pieces. How does the TAL1 complex connect enhancers to promoters in three-dimensional space and engage the transcriptional machinery? Tan et al show that *ARIEL* seems to be a missing piece.

Tan et al report that the TAL1 complex and partners bind an enhancer at −135 kb from the transcriptional start site of *ARID5B* (see figure), which induces transcription of *ARIEL* from the enhancer element.[6] Accordingly, *ARIEL* has higher expression in TAL1-type T-ALL cells than other T-ALL cells. Several carefully performed assays (eg, 4C-seq, chromatin immunoprecipitation polymerase chain reaction [PCR], chromatin isolation by RNA purification PCR, RNA immunoprecipitation, and RNA pull-down) showed that *ARIEL* binds mediator (a transcriptional coactivator complex), the *ARID5B* promoter, and the −135-kb enhancer. To test whether *ARIEL* is essential for assembling the *ARID5B* enhancer-promoter structure, Tan et al knocked *ARIEL* down. Loss of *ARIEL* reduced enhancer-promoter interactions and enhancer occupancy of TAL1 complex members, partner proteins, mediator proteins, RNA polymerase II, and ARID5B itself. Furthermore, *ARIEL* knockdown reduced the expression of *ARID5B* and TAL1 target genes and impaired leukemic proliferation in vitro and in vivo. In other words, take *ARIEL* away and large protein-DNA complexes fall apart, oncogenic transcriptional loops unravel, and leukemic cells die.

Importantly, the studies by Tan et al were rigorous. The use of multiple orthogonal methods raises confidence in the proposed dynamic structural effects of *ARIEL* on the *ARID5B* locus (see figure). Rigor was particularly evident when the authors showed that *ARIEL* had strong effects on some specific proteins and genes (but not others) and in some TAL1-positive cell lines (but not others). The discoveries of *LUNAR1* and *ARIEL* argue for a wider search for eRNAs that control T-ALL leukemogenesis. These elegant studies can serve as a basic framework for future experimental designs. However, there were limitations that should be noted. For example, despite numerous attempts, Tan et al were unable to test the potential of *ARIEL* as a therapeutic target through genetic silencing in primary patient-derived xenografts. These technical issues highlight a need to improve current protocols for widespread applicability.[9,10] Another limitation is that it remains unclear which effects of *ARIEL* are direct. Mapping the direct eRNA-to-protein or eRNA-to-DNA interactions is challenging but would be useful for designing potential inhibitors that disrupt eRNA functions.

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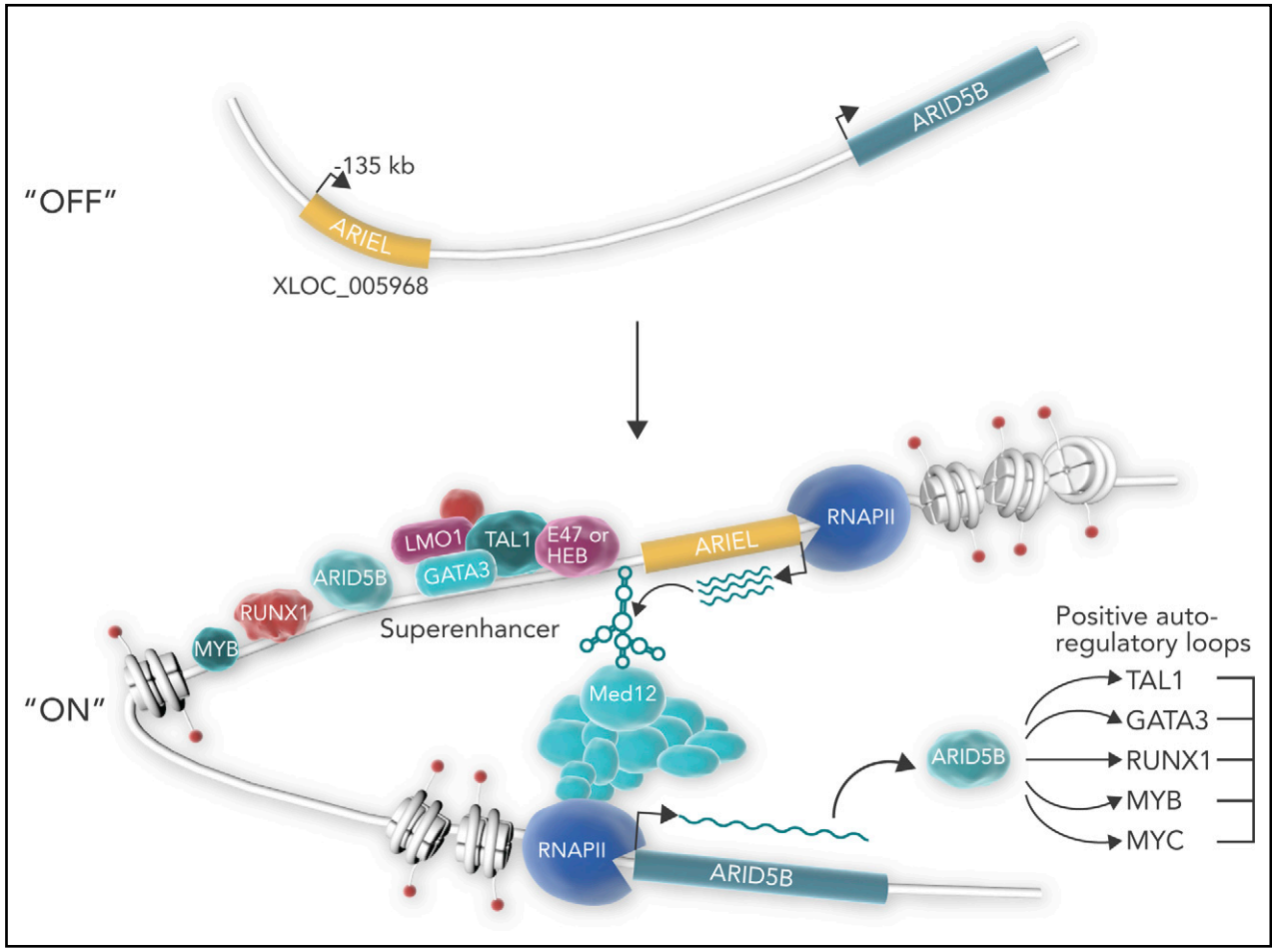

ARIEL (ARID5B-inducing enhancer-associated long noncoding RNA/*XLOC_005968*) acts as an eRNA in a subset of TAL1-type T-ALL. The TAL1 complex in T-ALL consists of several transcriptional regulators that include the class II basic helix-loop-helix transcription factor TAL1, E proteins (eg, E47 or HEB), LIM-only proteins (eg, LMO1/2), and GATA factors (eg, GATA3). This complex frequently co-occupies enhancers with partner transcription factors ARID5B, RUNX1, and MYB. In the nucleus, the TAL1 complex instructs RNA polymerase II (RNAPII) to transcribe *ARIEL* from the *ARID5B* enhancer template located at −135 kb relative to the *ARID5B* transcriptional start site.[1,6] *ARIEL* connects the enhancer to MED12, a subunit of the mediator coactivator complex that binds RNAPII. Thus, *ARIEL* facilitates chromatin looping interactions between the enhancer and the *ARID5B* promoter. These interactions in turn promote the expression of *ARID5B*, which amplifies positive autoregulatory loops that generate the TAL1 oncogenic transcriptional program.[7,8] Upregulated expression of ARID5B target genes promotes *ARIEL* transcription. In this diagram, direct interactions involving *ARIEL* and genomic coordinates of factors are postulated.

Tan et al discovered a key mechanism and vulnerability that amplifies the TAL1-driven oncogenic genetic program. However, the clinical implications remain speculative. There is currently no strategy to effectively target eRNAs. Synthetic nucleotides such as antisense oligonucleotides and small interfering RNAs could be designed to silence *ARIEL* expression. However, for decades, use of synthetic nucleotides to target RNAs has met formidable obstacles. In particular, in vivo delivery of synthetic nucleotides into cancer cells has been challenging. Furthermore, the potential toxicity of *ARIEL* inhibition is unknown, but it could be addressed using genetically modified animal models. Finally, the potential of *ARIEL* as a biomarker remains to be explored. It is unknown whether its expression correlates with patient outcomes. Because the explosive growth of new reports elucidating eRNA biochemistry is expected to continue in the near future, it is possible that new strategies for clinical translation will soon be revealed.

Mechanistically, *ARIEL* (as well as other eRNAs) remains enigmatic. For example, *ARIEL* acts as a cis regulator of *ARID5B* but also seems to have yet-to-be-revealed ARID5B-independent effects. In addition, yet-to-be-revealed context-dependent factors seem to regulate *ARIEL* expression because many TAL1$^+$ T-ALLs do not express *ARIEL*. Finally, candidate approaches that test individual eRNAs have met with some success but have not generated the momentum needed to radically advance the field. It will be important to devise large-scale functional genomic studies to screen for oncogenic eRNAs. The ARID5B-activated circuit is only 1 part of a vast sea of circuits. Tan et al found *ARIEL*, but there are more leukemia-associated eRNAs to discover. For now, we eagerly wait to learn what other eRNAs lie under the sea of circuits that support T-ALL.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2019001584

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Comment on Ferreri et al, page 252

Is it time to revisit R-CHOP for primary CNS lymphoma?

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In this issue of *Blood*, Ferreri et al report preliminary results from the INGRID trial regarding the feasibility and safety of using engineered tumor necrosis factor-α for increasing blood-brain barrier permeability in patients with relapsed or refractory primary central nervous system (CNS) lymphoma.[1] The authors demonstrate that treatment with tumor necrosis factor coupled with NGR (NGR-hTNF), a drug that targets CD13 on tumor blood vessels, followed by rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is safe, selectively increases permeability of the blood-brain barrier (BBB) in the tumor regions, and is associated with radiographic responses.

Primary central nervous system lymphoma (PCNSL), a rare type of extranodal diffuse large B-cell lymphoma (DLBCL), is typically treated with methotrexate-based induction followed by consolidative whole-brain radiation therapy vs myeloablative or nonmyeloablative chemotherapy. However, survival of PCNSL patients is inferior to that of other extranodal lymphomas, and improved treatments are needed. In addition to the unique biological characteristics of lymphoma within the nervous system, resistance to therapy is also mediated by the inability to adequately deliver therapeutic agents to the cancer due to the BBB.[2] The BBB is a dynamic, complex structure of pericytes, astrocytes, and endothelial cells connected by tight junctions that regulate the biochemical composition of the brain interstitial milieu and protects the brain from toxic molecules (including xenobiotics). Because of its physicochemical properties, the BBB is relatively impermeable to many water-soluble compounds. Most cytotoxic drugs that gain access to the brain cross the BBB by passive diffusion. Aside from pharmacokinetic properties, the main factors that influence the extent to which these compounds distribute into the brain include lipid solubility, molecular mass, charge, and plasma protein binding. Specifically, small organic compounds with a molecular weight <200 that are lipid soluble, neutral at physiologic pH, and not highly bound to plasma proteins readily cross the BBB. Consequently, this limits delivery and cancer cell exposure of certain chemotherapeutic agents (including the components of R-CHOP), antibodies, and cell therapies. However, the BBB is partially disrupted in the setting of malignant neoplasms, including PCNSL. This partial disruption is the basis for the characteristic contrast leakage noted on brain computed tomography or magnetic resonance imaging studies and may demarcate a window during which there is enhanced delivery of chemotherapeutics. However, PCNSL is a diffuse disease with lymphoma cells infiltrating throughout the brain "behind" intact BBB, well beyond the contrast-enhancing borders noted on neuroimaging.[3] Moreover, there is evidence that partially disrupted BBB is reconstituted with treatment, as marked by decreased contrast enhancement on computed tomography or magnetic resonance imaging. To further complicate matters, because of concurrent involvement of the brain, cerebrospinal fluid (CSF), and the eye in up to 20% of cases, it is not only the BBB, but also the blood–CSF barrier and the blood–retinal barrier that are relevant for drug delivery in PCNSL.[4] Thus, strategies that enhance delivery of therapeutic agents beyond the BBB are critical for success.

Studies to date have demonstrated poor efficacy of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in primary CNS DLBCL despite its demonstrated efficacy in systemic DLBCL. Radiographic response rates to CHOP or cyclophosphamide, doxorubicin, vincristine, and dexamethasone in PCNSL are 19% to 59%.[5,6] However, the responses are not durable, with early progression in most patients. Moreover, single-arm studies demonstrated a median survival of only 10 to 18 months. A randomized phase 3 trial of whole-brain radiation therapy with or without CHOP demonstrated no apparent difference in survival between the 2 arms, although the trial was terminated early because of poor accrual.[7] Consequently, R-CHOP is not considered an effective induction regimen for PCNSL. Compromised delivery of the component drugs of R-CHOP beyond the BBB is likely an important factor in the failure of this regimen in PCNSL.

There have been considerable efforts to overcome the BBB and enhance drug delivery to brain tumors. Some of these strategies include direct drug administration into the brain using biodegradable polymers

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