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COMMENTARY

A commentary on types of DNA methylation status of the interspersed repetitive sequences for LINE-1, Alu, HERV-E and HERV-K in the neutrophils from systemic lupus erythematosus patients and healthy controls

Wesley Brooks

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Genetics and adaptive immunity have been the focus of most research in autoimmune diseases, but now there is growing interest regarding involvement of epigenetics and the innate immune response. The report by Hirankarn *et al.*[1] in this issue is quite timely as it provides insights into DNA methylation (epigenetic control) in neutrophils (first responders in the innate response) in lupus patients.

As neutrophils comprise the majority of white blood cells, are part of the innate immune response, and are typically the first immune cells to arrive at an infection site, we should suspect them of having an important role early in an autoimmune episode. Indeed, the recently discovered innate immune response process of NETosis has been proposed as a means by which modified nuclear material can be exposed extracellularly to the adaptive immune system and potentially initiate an autoimmune reaction.[2,3] In NETosis, a neutrophil is stimulated to modify its chromatin and extrude the chromatin as a neutrophil extracellular trap, which binds pathogenic material to facilitate rapid removal by macrophages.[4] As this process yields a mixture of pathogenic and nuclear material during an inflammatory reaction, it is important to consider how abnormal epigenetic changes could alter this process.

Hirankarn *et al.*[1] performed DNA methylation analysis of interspersed repetitive sequences (IRS): LINE-1, Alu, HERV-E and HERV-K in neutrophils comparing lupus patients against normal controls. IRS constitute ~45% of the human genome and are enriched in G–C methylation sites. Therefore, changes in IRS methylation can not only affect expression of IRS, but also have a large effect on neighboring genes and genes that contain IRS in their introns or exons, potentially leading to abnormal expression or suppression of these neighboring genes. Besides the potential local impact of hypomethylated IRS, some IRS copies have potential for retrotransposition, that is, IRS can be copied and inserted at other sites in the genome. This can include additional material being moved with the IRS, such as an exon from one gene being added to another gene facilitated by IRS retrotransposition.

This new report found no significant differences in DNA methylation of Alu, HERV-E and HERV-K sequences when they compared lupus patients against normal controls. However, the results showed a significant decrease in methylation of some LINE-1 sequences (L1s) in lupus patients compared with normal controls. Many of the hypomethylated L1s were intragenic and antisense to a larger gene. They observed some genes with upregulation and other genes with downregulation when the intragenic L1 was hypomethylated. No significant differences were seen in L1 methylation when comparing active and inactive lupus based on SLEDAI-2K assessments of patients. This suggests there are other events involved in active lupus in addition to L1 hypomethylation. In previous work, which dealt with lymphocytes of the adaptive immune response, these researchers found L1 hypomethylation in T lymphocytes (CD4+, CD8+) and B lymphocytes in lupus patients versus controls.[5,6]

There are an estimated 500 000 L1s in the human genome. L1s are believed to have originated from a functional retrotransposon with two open reading frames (ORFs): ORF1, coding an RNA chaperone, and ORF2, coding for reverse transcriptase and endonuclease activity. Most L1s have become nonfunctional but an estimated 100 copies of 'young' L1s still retain functionality.[7]

There are important aspects to consider with regards to L1 hypomethylation. Bear in mind that the extent of hypomethylation and which L1s (young or old) can vary from neutrophil to neutrophil and, therefore, consequences can vary. In addition, consequences may not appear until activation of the neutrophil in an innate immune response.

First, L1 hypomethylation can impact local epigenetic control. Hypomethylation can lead to L1 expression, which can aid in opening neighboring genes, possibly with L1 acting as a secondary initiation site for those genes. Alternatively, L1 in antisense strands can create RNA transcripts that hybridize with sense strand transcripts leading to double-stranded RNA degradation by the RNA-induced silencing complex (RISC), thereby suppressing gene expression.

Second, L1 hypomethylation can impact broader epigenetic control. X-chromosome inactivation (XCI) is an epigenetic process in which one of the two X chromosomes in

W Brooks is at Department of Chemistry, University of South Florida, Tampa, FL, USA
E-mail: wesleybrooks@mindspring.com

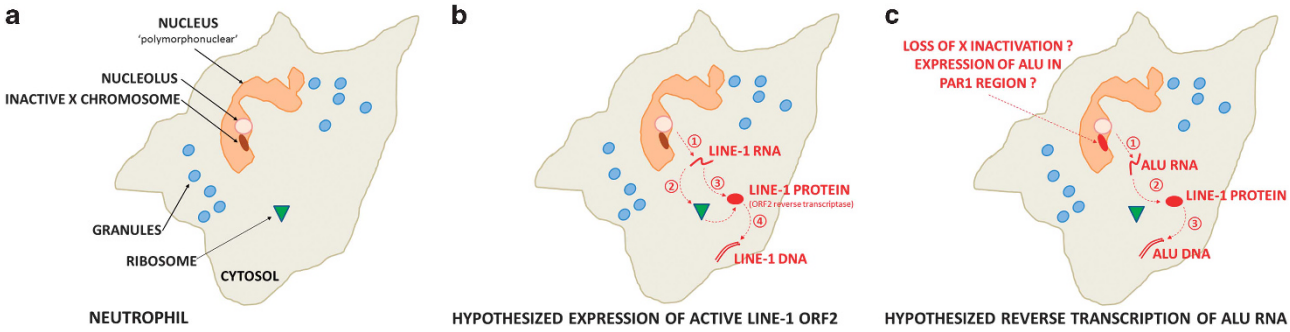

Figure 1 Possible impact of functional LINE-1 expression in neutrophils. (**a**) Neutrophils are known as granulocytes due to the presence of granules containing degradative enzymes, antibacterial peptides and chromatin-modifying enzymes used in NETosis or phagocytosis. Neutrophils are also known as polymorphonuclear leukocytes because of their oddly shaped nuclei. The inactive X chromosome is a dense appearing heterochromatic body in the nucleus, typically perinuclear and often found in close proximity to the nucleolus. LINE-1 sequences (L1s) are believed to have a role in X-chromosome inactivation (XCI). (**b**) Hypomethylation of L1s could lead to ① transcription and ② translation into LINE-1 protein. Functional LINE-1 ORF2 reverse transcriptase could create ③ and ④ LINE-1 DNA. (**c**) Loss of XCI could open Alu sequences for ① transcription and ② reverse transcription by LINE-1 protein creating ③ hypomethylated Alu DNA.

each female cell is epigenetically silenced as most X-linked genes are not sex-specific and need only one active copy. This equilibrates X-linked expression in male and female cells giving what is termed dosage compensation. The resulting inactive X (Xi) is a dense heterochromatic structure with about 85% of Xi genes silenced, but this can vary. L1s make up 30% of the X chromosome and are 2× enriched on the X compared with autosomes. In addition, distribution of L1s on the X parallels the intensity of silencing. These facts led Mary Lyon, originator of the X inactivation concept, to propose the Lyon repeat hypothesis, in which L1s are anchoring sites involved in the spread of XCI.[8] It is conceivable that L1 hypomethylation on the Xi could initiate reactivation of previously silenced genes in a few neutrophils, which then overexpress some X-linked genes. This potential loss of X-linked dosage compensation could contribute to female bias of autoimmune diseases. Particularly interesting is a high concentration of Alu elements in the PAR1 region of the X short arm.

Third, hypomethylation of L1s that still have ORF2 endonuclease functionality could lead to aberrant cutting of DNA, disrupting the chromatin. This could potentially involve retrotransposition events or trigger apoptosis.

Fourth, hypomethylation of L1s that still have ORF2 reverse transcriptase functionality could lead to their expression and reverse transcriptase activity (Figure 1). Dewannieux *et al.*[9] reported a 1000× preference of L1 reverse transcriptases for L1 RNA compared with other RNAs and a 300× preference for Alu RNA. This could occur due to close proximity of L1 protein to L1 RNA at the ribosome and Alu sequences in transcripts, such as the Alu domain of the signal recognition particle that regulates ribosome activity. Free DNA in lupus patient sera has been reported to be as much as 55% Alu DNA.[10] This suggests the hypothesis that, in a few neutrophils, active lupus may arise from functional L1 reverse transcriptase activity and an abundance of Alu RNA that get reverse transcribed.[11]

1 Sukapan, P., Promnarate, P., Avihingsanon, Y., Mutirangura, A. & Hirankarn, N. Types of DNA methylation status of the interspersed repetitive sequences for LINE-1, Alu, HERV-E and HERV-K in the neutrophils from systemic lupus erythematosus patients and healthy controls. *J. Hum. Genet.* **59,** 178–188 (2014).
2 Su, K. Y. & Pisetsky, D. S. Role of extracellular DNA in autoimmunity in SLE. *Scand. J. Immunol.* **70,** 175–183 (2009).
3 Brooks, W. H. Increased polyamines alter chromatin and stabilize autoantigens in autoimmune diseases. *Front. Immunol.* **4,** 91 (2013).
4 Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S. *et al.* Neutrophil extracellular traps kill bacteria. *Science* **303,** 1532–1535 (2004).
5 Nakkuntod, J., Avihingsanooin, Y., Mutirangura, A. & Hirankarn, N. Hypomethylation of LINE-1 but not Alu in lymphocyte subsets of systemic lupus erythematosus patients. *Clin. Chim. Acta* **412,** 1457–1461 (2011).
6 Nakkuntod, J., Sukkapan, P., Avihingsanon, Y., Mutirangura, A. & Hirankarn, N. DNA methylation of human endogenous retrovirus in systemic lupus erythematosus. *J. Hum. Genet.* **58,** 241–249 (2013).
7 Hancks, D. C. & Kazazian, H. H. Active human retrotransposons: variation and disease. *Curr. Opin. Genet. Dev.* **22,** 191–203 (2012).
8 Chow, J. C., Ciaudo, C., Fazzari, M. J., Mise, N., Servant, N., Glass, J. L. *et al.* LINE-1 activity in facultative heterochromatin formation during X chromosome inactivation. *Cell* **141,** 956–969 (2010).
9 Dewannieux, M., Esnault, C. & Heidmann, T. LINE-mediated retrotransposition of marked Alu sequences. *Nat. Genet.* **35,** 41–48 (2003).
10 Li, J. Z. & Steinman, C. R. Plasma DNA in systemic lupus erythematosus. Characterization of cloned base sequences. *Arthritis Rheum.* **32,** 726–733 (1989).
11 Brooks, W. H. Systemic lupus erythematosus and related autoimmune diseases are antigen-driven, epigenetic diseases. *Med. Hypotheses* **59,** 736–741 (2002).