The enemy within: dormant retroviruses awaken

Michael E Engel & Scott W Hiebert

Mammalian genomes harbor regulatory elements from ancient retroviral infections. These retroviral remnants are normally silenced by DNA methylation—but this can change. Reactivation of one such element triggers the expression of a nearby oncogene during the development of Hodgkin's lymphoma (571–579).

Over 100 million years ago, retroviruses began their assault on mammals by infiltrating the mammalian genome. In defense, mammals developed the ability to recognize and silence transcription from these integrated retroviruses. Through evolutionary time, the remnants of this conflict have accumulated in our DNA. Conservatively, 7–8% of the present-day human genome is derived from retroviral and retrotransposon sequences[1], yet the impact of these "ancient scars" on host gene expression and their contribution to human disease remain unanswered questions.

The study by Lamprecht *et al.*[2] in this issue of *Nature Medicine* offers insights into these fundamental concerns. The authors find that aberrant expression of the protooncogene *CSF1R* (encoding colony-stimulating factor-1 receptor) in human lymphomas is driven by activation of a nearby, endogenous long terminal repeat (LTR) that is normally silenced[2].

The retroviral RNA genome is reverse transcribed into a DNA provirus, which integrates into the host genome[3]. The ends of the retroviral genome contain repetitive elements known as LTRs, which recruit host transcription factors to serve as promoters and enhancers for retroviral gene transcription. However, these elements can also enhance the expression of neighboring host genes. Because growth and development require high-fidelity host gene expression, cells must either accommodate LTRs or achieve dominance over them.

Mammalian cells limit the functions of retroviral LTRs primarily through methylation of DNA at CpG dinucleotides[4,5]. Genome-wide CpG methylation is established at implantation in vertebrate embryos and favors transcriptional silencing. As development proceeds, discrete regions within the genome are demethylated in a cell type–specific manner, generating patterns of gene expression specific to a particular lineage. However, inappropriate gene expression might occur if CpG methylation is reversed on silenced, endogenous retroviral LTRs.

Lamprecht *et al.*[2] have addressed the importance of inappropriate gene expression in the survival of cells from individuals with Hodgkin's lymphoma, a malignancy of the B lymphocyte lineage.

The authors examined Hodgkin's lymphoma cell lines and primary samples from affected individuals and observed expression of the myeloid-specific genes encoding colony-stimulating factor (*CSF1*) and its receptor (*CSF1R*). Engagement of CSF1R by CSF stimulates cell proliferation via the Ras signaling pathway, so inappropriate expression of CSF1 and CSF1R in Hodgkin's lymphoma cells may establish an autocrine loop to stimulate lymphoma growth. In support of this hypothesis, Hodgkin's lymphoma cells underwent apoptosis when CSF signaling was blocked.

Further experiments revealed that *CSF1R* expression was activated in these cells by the reactivation of an upstream endogenous retroviral element. *CSF1R* was expressed in both myeloid and Hodgkin's lymphoma cell lines, but the native *CSF1R* promoter was not used in Hodgkin's lymphoma cells, suggesting that transcription initiated from a distinct site. The elongated *CSF1R* transcripts in Hodgkin's lymphoma cells instead originated from an LTR of the mammalian apparent LTR retrotransposon (MaLR) *THE1B* family—a subfamily that makes up only a small minority of the endogenous retroviruses[1]—situated 6.2 kb upstream of the normal *CSF1R* transcription start site.

The LTR was demethylated and transcriptionally active in Hodgkin's lymphoma cells but silenced by CpG methylation in peripheral blood mononuclear cells, non-Hodgkin's lymphoma cells and cells of the myeloid lineage (**Fig. 1**). Moreover, this was not an isolated event, as activation of *THE1B* LTRs that are distributed throughout the genome was seen specifically in Hodgkin's lymphoma cell lines.

Collectively, these findings provide compelling evidence that retroviral LTRs lying dormant in the mammalian genome can be awakened to direct aberrant gene expression in a human disease.

Mechanistically, the authors[2] present provocative early data to suggest that *THE1B* LTR silencing is controlled by the transcriptional co-repressor *CBFA2T3* (also known as MTG16 or ETO2). *CBFA2T3* encodes a transcriptional co-repressor that acts to bridge DNA binding proteins to epigenetic modifiers such as histone deacetylases to repress gene expression[6].

In non-Hodgkin's lymphoma cells, where *CSF1R* is repressed, LTR-driven *CSF1R* transcription could be synergistically induced by inhibiting DNA methyltransferases and class I histone deacetylases (using 5-aza-2'-deoxycytidine and trichostatin-A, respectively), suggesting that epigenetic mechanisms control the LTR. A survey to detect altered expression among epigenetic modifiers revealed reduced or absent expression of *CBFA2T3* in Hodgkin's lymphoma cells. Reduced expression was attributed to CpG methylation of the *CBFA2T3* locus. Notably, siRNAs directed toward *CBFA2T3* allowed LTR-driven *CSF1R* transcription in cells where *CSF1R* is otherwise silent.

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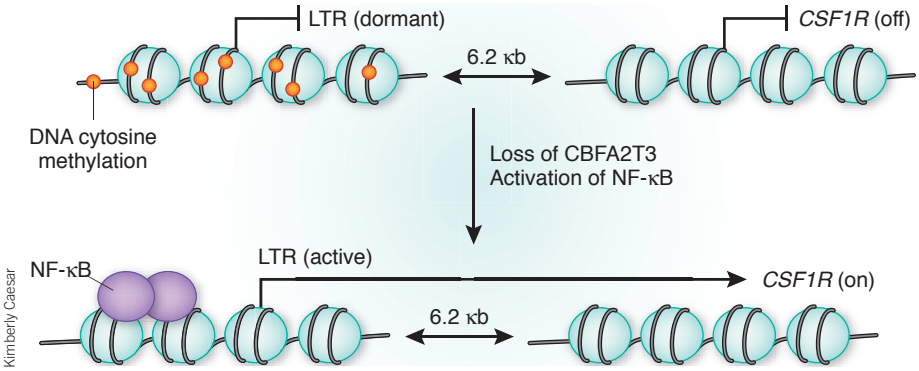

Figure 1 Aberrant expression of CSF1R is driven by the LTR of an endogenous retrovirus in Hodgkin's lymphoma. The upper portion of the schematic diagram shows a dormant retroviral sequence that is silenced by DNA methylation. With loss of *CBFA2T3* expression and activation of NF-κB, the LTR is reactivated to produce an mRNA that is transcribed through the *CSF1R* locus.

The authors went on to show synergistic effects on gene expression when they combined *CBFA2T3*-specific siRNA treatment with activation of nuclear factor-κB (NF-κB)[2]. NF-κB is often constitutively active in Hodgkin's lymphoma, and NF-κB response elements are conserved within *THE1B* LTRs. Thus, when *CBFA2T3*-mediated silencing of the *THE1B* LTR is reversed, NF-κB activation stimulates CSF1R expression.

In Hodgkin's lymphoma, the reactivation of a latent LTR permits CSF1R signals needed for tumor cell survival. However, these silenced sequences are spread throughout our genomes. Might genome-wide awakening of dormant transcription contribute to the development of a tumor and its clinical manifestations in other ways? Could these LTRs affect expression of other oncogenes? Perhaps these endogenous viral sequences affect the expression of noncoding RNAs that interfere with the expression of tumor suppressors or affect miRNA expression.

It is noteworthy that conventional microarray analyses will not detect aberrant transcriptional start sites, but deep sequencing of tumor RNA should reveal the extent of LTR reactivation. The identification of structurally distinct transcripts driven by retroviral LTRs may provide new options for the diagnosis and monitoring of disease or perhaps uncover new therapeutic opportunities.

The findings of Lamprecht *et al.*[2] could also have wide-ranging implications for other cancer types and for other diseases. For example, *CBFA2T3* is disrupted by the t(16;21) translocation in myeloid leukemias[7], and it is situated at 16q24.3, which is frequently deleted in breast tumors (59% of primary breast tumors showed reduced or absent *CBFA2T3* expression[8]). Furthermore, NF-κB activation is common in many tumors—do these tumors show signs of reactivation of the *THE1B* LTRs? This work may also stimulate the hunt for *THE1B* reactivation in other diseases that are characterized by changes in the patterns of DNA methylation.

There are several thousand endogenous retroviral insertions in the human genome[9], suggesting that loss of control of these elements may contribute to a wide range of human diseases. Mammalian genomes have evolved to minimize the potential deleterious effects of these insertions, and in rare circumstances to exploit them. The new findings reveal the wisdom of this design and draw attention to the consequences of system failures[2].

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Fighting off pain with resolvins

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Pain caused by chronic inflammation is a serious health problem. But the currently available analgesic drugs cause major side effects when taken long term. A new study points to a class of molecules, resolvins, which not only provide analgesia and are well tolerated but may also reduce inflammation (pages 592–597).

Local anesthetics reduce normal sensation as well as pain. But when it comes to treating chronic pain, the ideal medication should provide analgesia without altering normal sensation. In addition, it should be free of unwanted side effects, in particular organ damage, and, ideally, not only act upon the symptom of pain but also ameliorate the underlying condition causing the pain. In this issue of *Nature Medicine*, Xu *et al.*[1] report a group of molecules belonging to the resolvin family that have the potential to become such an ideal analgesic drug, at least in the context of inflammatory pain.

Pain caused by chronic inflammation, such as that in arthritis, lower back pain or inflammatory bowel disease, causes suffering and high health care and socioeconomic costs. People with chronic inflammatory pain usually take medications such as inhibitors of cyclooxygenase (COX) enzymes or opioids. But long-term use of these drugs is not without problems. Unselective COX inhibitors may cause gastrointestinal bleeding and damage to kidneys, whereas more selective COX2 inhibitors increase the risk of cardiovascular disease[2]. Although opioids are strong and effective analgesics for treating acute pain, long-term use may require increasing doses and cause unwanted side effects, such as drowsiness, constipation, nausea, sedation and cognitive

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