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RESEARCH HIGHLIGHT

A clear link between endogenous retroviral LTR activity and Hodgkin's lymphoma

Katryn J Stacey[1], Vitaliya Sagulenko[1]

[1]*The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Qld 4072, Australia*
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Abnormal activity of an endogenous retroviral promoter plays a critical role in the development of Hodgkin's lymphoma, according to a paper in the May issue of *Nature Medicine* [1]. Throughout evolution, infection of germline cells with retroviruses has led to the progressive accumulation of endogenous retroviral elements in genomes. The infecting retroviral RNA is reverse transcribed into DNA which inserts into the genome and is then transmitted to off-spring in a Mendelian manner. There are approximately 443 000 endogenous retroviral loci in the human genome, comprising 8% of the total DNA [2]. Most of these contain multiple mutations rendering them non-infectious, and there are many instances of isolated long terminal repeat (LTR) viral promoter elements rather than complete pro-viral sequences. These invading sequences have provided raw materials for the evolution of genomes. A prominent example of this is the expression of an endogenous retroviral-derived *env* gene product, syncytin, which is required for the fusion of trophoblasts to form a syncytium in the placenta [3]. More frequently, inserted LTRs provide alternative promoters, enhancers, splice sites, poly A signals, or drive antisense regulatory transcripts [4]. However, invading DNA elements present a risk for the organism, and the majority are silenced by methylation [5].

The inappropriate activation of endogenous retroviruses has been observed in a number of different cancers, although evidence of a direct causal role in tumour progression has been lacking [6]. However, the study by Lamprecht and colleagues [1] clearly shows the role of an endogenous retroviral LTR in development of Hodgkin's lymphoma. Hodgkin's lymphoma cells originate from mature B cells, but were demonstrated to require autocrine signaling by the macrophage growth factor, colony stimulating factor 1 (CSF1) for proliferation and survival [1]. The receptor for CSF1 (CSF1R), a proto-oncogene product, is normally expressed in cells of the macrophage, osteoclast and trophoblast lineages. The lineage-inappropriate transcription of the *CSF1R* (*c-fms*) gene in Hodgkin's lymphoma cells was not initiated from the canonical macrophage *CSF1R* promoter but from an LTR element of the "mammalian apparent LTR retrotransposon" (MaLR) *THE1B* family, located 6.2 kb upstream (Figure 1). This aberrant LTR-driven *CSF1R* transcript was detected in lymph nodes from all tested Hodgkin's lymphoma patients and several samples of anaplastic large cell lymphoma, but not in primary samples of other types of lymphoma.

The essential role for CSF1/CSF1R autocrine signaling in Hodgkin's lymphoma was demonstrated using soluble decoy CSF1R which reduced proliferation by approximately 60%, and CSF1R inhibitors which strongly reduced viability of Hodgkin's lymphoma cells. Caution is needed in interpretation of inhibitor studies, since the inhibitor used may also affect related tyrosine kinase receptors such as KIT and PDGFR [7]. However, such inhibitors are promising for treatment of Hodgkin's lymphoma.

Activation of *THE1* LTRs in Hodgkin's lymphoma cells was not restricted to the *CSF1R* locus, but was widespread, indicating a general loss of repression of these elements. The LTR promoter activity was strongly dependent on binding sites for NF-κB, AP-1 and Sp1 transcription factors. These sites were conserved among *THE1* subfamily LTRs, and the constitutive NF-κB and AP1 activity found in Hodgkin's lymphoma cells [8] is no doubt important in driving the observed promoter activity. The normal widespread suppression of LTR activity by methylation [5] pointed to a loss of epigenetic control in Hodgkin's lymphoma cells. The authors identified two CpG elements in the LTR sequence which were strongly demethylated in Hodgkin's lymphoma cells. In contrast, these were at least partially methylated in many other lymphoma and leukemia cells, and almost fully methylated in peripheral blood cells of healthy donors. In support of perturbed epigenetic modifications

Correspondence: Katryn J Stacey
E-mail: katryn.stacey@uq.edu.au

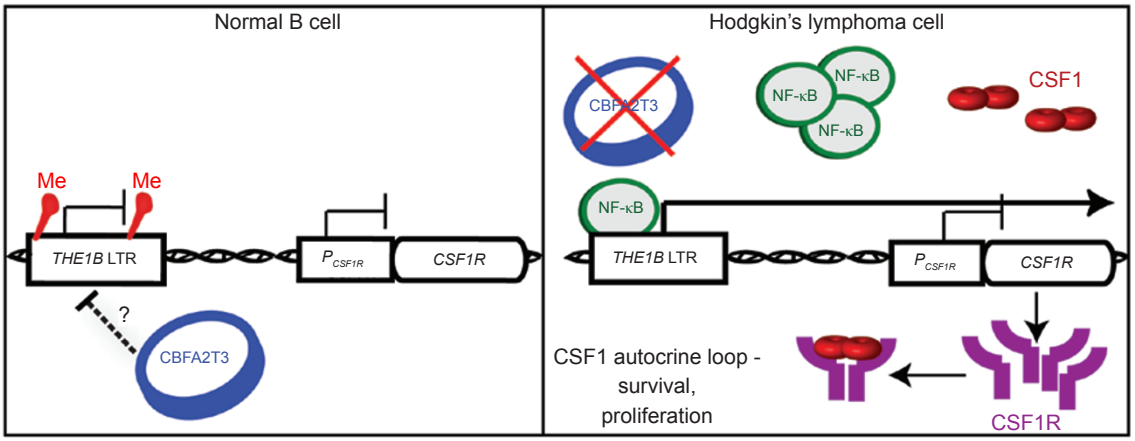

Figure 1 Derepression of an LTR sequence 6.2 kb upstream of the normal macrophage promoter (P_{CSF1R}) is responsible for expression of the *CSF1R* gene in Hodgkin's lymphoma cells. In normal B cells, the LTR is CpG methylated (Me) and inactive. Hodgkin's lymphoma cells have constitutive NF-κB transcription factor activity and loss of transcriptional repressor CBFA2T3, which leads to LTR promoter activity. The normal role of CBFA2T3 in suppressing LTR activity may be indirect. CSF1R expression allows autocrine proliferative and survival signals in Hodgkin's lymphoma.

being responsible for LTR activation, treatment with 5-aza-deoxycytidine to reduce CpG methylation levels and a histone deacetylase inhibitor induced LTR-dependent CSF1R expression in cells that did not normally express CSF1R.

Mechanisms involved in the loss of epigenetic control of *THE1* LTRs in Hodgkin's lymphoma are not clearly understood, but are likely to involve CBFA2T3, a transcriptional repressor of the ETO family which normally acts via interaction with histone deacetylases and co-repressor factors. The authors found CBFA2T3 was constitutively expressed in normal B cells and non-Hodgkin's lymphomas but was strongly downregulated in Hodgkin's lymphoma cells. RNAi-mediated suppression of CBFA2T3 together with induction of chronic NF-κB activity were able to induce the aberrant LTR-driven *CSF1R* transcript in a leukemic cell line. The cell line used normally has a high level of CpG methylation of the *THE1* LTR, but no analysis of methylation status following this treatment was presented. The authors note that enforced expression of CBFA2T3 in Hodgkin's lymphoma cells was not able to inhibit LTR promoter activity, suggesting that CBFA2T3 alone could not reverse the chromatin modeling at the active LTR. Taken together, the data suggests that activation of *THE1* LTRs in Hodgkin's lymphoma depends on both constitutive NF-κB and epigenetic or mutational silencing of CBFA2T3. The precise role of CBFA2T3 in suppression of the *THE1* LTRs remains to be established.

There are several important implications of this work. Whilst many different epigenetic changes have been implicated in cancer, this is the first demonstration of demethylation of an endogenous retroviral LTR allowing expression of a proto-oncogene. This nicely illustrates the importance of cellular mechanisms designed to silence invading DNA elements. There may well be more examples of LTR-driven genes contributing to cancer. Secondly, CSF1R expression has been detected in a range of tumors of epithelial origin (see references in [9]), and it will be interesting to determine if there is similar loss of epigenetic control of the *THE1* LTR in any of these cancers. Lastly, CSF-1R signaling is a promising therapeutic target in Hodgkin's lymphoma and any other malignancy where CSF1R contributes to cancer cell growth and survival.

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