Epigenetic control of retrotransposons in adult tissues: Implications for immune regulation

Running title: **Epigenetic control of retrotransposons in adult tissues**

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Abstract

Retrotransposons tune immune reactivity in differentiated cells because when they are transcribed, their nucleic acids can be viewed as non-self leading to innate immune sensing. Most retrotransposons, however, are subject to transcriptional regulation by a multitude of epigenetic pathways, which have coevolved with them for millions of years. While a lot is known about the epigenetic control of retrotransposons in germ cells and early embryos, surprisingly little is understood about these pathways in adult tissues, particularly in human cells. Recent evidence suggests that retrotransposon repression persists in differentiated cells and is dynamic. Future insight into this topic may teach us how to reactivate or silence specific retrotransposon families, to promote anti-tumour immunity or dampen autoimmunity through epigenetic modulation.

Introduction

Transposable elements (TEs) are mobile genetic elements aptly named "controlling elements" by Barbara McClintock in the 1950s because of their ability to control cellular genes [1]. Since their discovery, the advancement of genome sequencing and bioinformatics technologies has led to the identification of a huge number of TE families, the functions of which are now being unraveled. Retrotransposons are particularly relevant TEs because they can replicate through an RNA intermediate, allowing them to insert new DNA copies of themselves into the genome. This has enabled them to accumulate over millions of years and they now comprise more than half of the human genome[2]. Over

time, deleterious insertions are negatively selected while those beneficial to the host become co-opted and fixed in the genome[3]. Co-opted retrotransposons are often from the endogenous retrovirus (ERV) class, which dates back hundreds of millions of years [4]. Although retrotransposons are overall beneficial as a driving force behind the evolution of new genes and non-coding DNA[5, 6], they can also compromise genome and transcriptome integrity [7]. A multitude of epigenetic pathways, therefore, act in early development to constrain their transcription and some of these strategies remain active in differentiated cells.

Epigenetics refers to modifications on chromatin, rather than DNA sequence alterations, which lead to heritable effects on gene expression. Chromatin is subject to histone modifications and cytosine methylation and distinct epigenetic marks are associated with an active and silent gene expression state. For example, acetylation of histone 3 at the lysine residue at position 27 (H3K27ac) is a chromatin signature associated with active genes and enhancers[8], whereas trimethylation of histone 3 at the lysine at position 9 (H3K9me3) correlates with heterochromatin and gene silencing[9-11]. Retrotransposons direct both genetic and epigenetic heritable traits because they can integrate into our genome in the germ line and orchestrate epigenetic alterations through the recruitment of transcription factors to their regulatory elements [12].

Since half of the human genome is derived from retrotransposons, it can be viewed as non-self. Although most human retrotransposons are no longer mobile, expression of their nucleic acids and proteins can lead to the formation of pathogen-associated molecular patterns (PAMPs) and antigens that we refer to here as “neo-antigens” that could potentially elicit an immunological response. Retrotransposons, are therefore situated at the interface of immune reactivity; when enriched in silent chromatin they are transcriptionally inactive and immune masked, whereas when expressed they may trigger innate and adaptive immunity [13-15]. In this review, we will discuss the mechanisms in place to maintain retrotransposons silent in differentiated cells and the implications of these pathways. We will focus here on chromatin readers, writers and erasers

and the KAP1 and KRAB-ZNF system. The role of small RNAs, while important, is beyond the scope of this review and we direct the reader to a recent review covering this topic[16].

Epigenetic pathways constraining retrotransposons

Epigenetic silencing of retrotransposons takes place in early embryos and in differentiated tissues, epigenetic states link back to patterns established during development[17]. The identification of factors that maintain repression in postembryonic tissues and how dynamic chromatin marks are in differentiated cell types is a fascinating and emerging area of research. Moreover, existing work relies largely on mouse models so it will be crucial to establish parallels in human cells, where the precise retrotransposons, their activity and their coevolution patterns with genes are distinct[18].

Chromatin readers, writers and erasers

Chromatin-associated proteins induce epigenetic changes to either histones or DNA and mediate downstream biological functions. Promoter cytosine methylation occurs at ERVs and is associated with gene repression [19, 20], whereas intragenic methylation prevents spurious transcription initiation [21]. Interestingly, human ERV-K (HERV-K) genomic sequences have undergone selection to mutate CpG dinucleotides, presumably to escape repression through DNA methylation [22]. *De novo* methylation is carried out by DNMT3A, DNMT3B, the newly discovered rodent-specific DNMT3C, and the cofactor DNMT3L [23-26] and maintained by DNMT1 through cell divisions [27, 28]. Defective DNMT1 leads to DNA hypomethylation and ERV overexpression and plays a causative role in the onset of cancer and autoimmune disease [29, 30]. Interestingly, the reactivation of ERVs observed following treatment of cells with DNA-demethylating agents, such as 5-azacytidine (5-AZA) [31] has been proposed to be responsible for driving anti-tumour immunity in cancer patients treated with these drugs through innate sensing of ERV nucleic acids [13, 14]. 5-AZA drugs are thought to target mainly DNMT3A and DNMT3B, which can be increased in expression upon differentiation [32], in contrast to DNMT3L, which is not expressed in differentiated cells [33].

Histone methyltransferases (HMTs) including SETDB1 (also known as ESET), SUV39h and G9a (also known as EHMT2) mediate retrotransposon repression through histone methylation [9, 10, 34]. The most relevant to this review is SETDB1 because it is required for retrotransposon repression in postembryonic tissues [35, 36]. Indeed, the finding that SETDB1 represses ERVs in committed mouse B-lineage cells, has led to a new paradigm that SETDB1 and potentially other histone modifiers remain important in differentiated cells [35]. Interestingly, loss of silent chromatin at SETDB1-regulated ERVs is not sufficient for their activation and the precise panel of ERVs reactivated in specific cell types depends on the transcription factors available [35]. HP1 too, which interacts with H3K9me3 [37, 38] and participates in heterochromatin spreading [39] has been implicated in silencing of ERVs including in differentiated cells [40, 41].

The KAP1 and KRAB-ZNF repertoire

The KAP1 and KRAB-ZNF (KRAB-zinc finger protein) system silences retrotransposons in early embryos and embryonic stem cells (ESCs) [12, 42, 43]. It has likely evolved in response to retrotransposon invasions [44] because KRAB-ZNF transcription factors are largely specific for transposon sequences [45-47] but now this pathway also participates in gene regulation [45, 48, 49]. KAP1 is recruited to transposons through the interaction of its RING, B-box and coiled–coil (RBCC) domain with the KRAB domain of KRAB-ZNFs. Transcriptional repression is mediated through co-factors such as SETDB1[50-52], which prevents binding of transcriptional activators[53, 54]. There are several hundred KRAB-ZNFs [55], which can be viewed as a panel of effector proteins specific for foreign DNA in the same way that an antibody repertoire is specific for foreign antigens. While many KRAB-ZNFs have recently been matched to their target sequences through chromatin immunoprecipitation experiments [45-47], only a few have been functionally characterized. These include human ZNF91 and ZNF93, which recognize specific SVA and LINE1 subfamily sequences, respectively [56]. The KAP1 and KRAB-ZNF pathway is functional in human and mouse ESCs and neural progenitor cells [42, 57-59] but little is known about its role in differentiated cells. However, one study showed

that KAP1 binds to certain ERV-K elements in human primary CD4+ T cells [59]. It was also reported that KAP1 and KRAB-ZFPs bind to several ERVs in mouse liver. Interestingly, while KAP1 knockout in the liver had little impact on the expression of these ERVs, several co-regulated cellular genes were affected [48]. This suggests that redundant mechanisms may converge to silence ERVs in differentiated cells [48]. Surprisingly, HSP90 has recently been implicated in the formation of a KAP1 repressor complex at ERVs [60]. Like KAP1, HSP90 is necessary to maintain silent chromatin at ERVs and prevent aberrant transcription of genes close to the ERVs that it regulates [49, 60]. Most interestingly, this is true not only in ESCs but also in differentiated macrophages [60]. The nucleosomal and remodeling deacetylase (NuRD) complex, which interacts with KAP1 has also been implicated in retrotransposon repression in differentiated cells [61].

Of note, not all ERV-derived sequences are subject to epigenetic silencing as some have been co-opted because their non-coding DNA regulatory elements, nucleic acids or even gene products benefit their hosts [5, 6, 62, 63]. Whether co-opted ERVs are subject to spatial or temporal repression by KAP1 remains an open question. However, certain KRAB-ZNFs have been found to bind to co-opted ERVs and to recruit transcriptional activators and pioneer factors, suggesting that these ZNFs function to switch on rather then switch off certain gene networks [45, 47].

Implications for immune regulation

ERVs have been implicated in multiple cancers and autoimmune diseases, including ovarian and breast cancer, systemic lupus erythematosus and multiple sclerosis [64, 65]. There is some convincing evidence that ERVs can play a causative role in cancer when their LTRs escape epigenetic repression [66] and interestingly, this involves mainly primate-specific ERVs [67]. For example, cryptic enhancers and promoters that reside within ERVs can drive expression of oncogenes[66, 68].

ERVs regulate the immune system in several ways. For example, double stranded RNA (dsRNA) produced from retrotransposons, following treatment of cancer cells with DNA methyltransferase inhibitors activates interferon through MDA5, MAVS and IRF7 [13, 14]. Cytoplasmic DNA resulting from reverse transcription also serves as an additional PAMP because it is detected by the cytosolic DNA sensor cGAS to activate type I interferon through STING [63, 69-72]. Likewise, Toll-like receptors contribute to ERV nucleic acid sensing [73]. Mutations within genes involved in nucleic acid metabolism including TREX1 are associated with autoimmune diseases [74], although such factors block classes of retrotransposons that are constitutively transcribed rather than those embedded within silent chromatin. In addition to innate immunity, ERVs can stimulate adaptive immunity too because their encoded gene products, which are necessary for their mobilization are subject to standard antigen processing and presentation pathways. For example, tumour-associated antigens (TAAs) can be derived from HERV-K envelope protein[75]. Such neo-antigens can evoke adaptive T cell and antibody responses [76], both of which have been demonstrated to regulate ERVs [15, 75].

Overall, ERV regulatory sequences including solo LTRs have been described to contribute to cancer by driving oncogenes, whereas longer ERVs if resurrected (for example through 5-AZA treatment) may promote anti-tumour immunity through their nucleic acids and proteins. Exactly how sensing of ERV nucleic acids leads to anti-tumour immunity is not fully understood but remarkably, it has been shown that if cancer initiating cells are pretreated with 5-AZA drugs before their injection into mice, they form less tumours and this phenotype is dependent on MAVS[14]. Likewise, if B16 melanoma cells are pretreated with 5-AZA before their injection into mice that receive anti-CTLA-4, they can stimulate complete tumour clearance[13]. Furthermore, interferon-responsive genes are upregulated in cancer patients treated with 5-AZA[77]. Interferon signaling is important presumably to promote apoptosis of cancer cells and to help to recruit cytotoxic T cells recognizing neo-antigens and other immune effectors to clear the tumour. It has also been shown that cytosolic RNA and DNA sensing of ERVs is necessary to induce T-independent B cell responses in mice[63]. This latter

work illustrates that ERVs have coevolved with their hosts to play a natural role in modulating the immune system. Overall, ERVs lie at the intersection of innate and adaptive immunity, due to their intrinsic immunogenicity.

Concluding remarks

While it was previously thought that histone marks are primarily required to silence retrotransposons only early in development[10, 50, 78, 79], where DNA methylation is reprogrammed[80, 81], recent evidence has led to a new paradigm whereby diverse epigenetic modifiers exert continuous roles in adult tissues. Here we discuss evidence that SETDB1, DNMTs, HP1, HSP90, the NuRD complex and potentially KAP1 and KRAB-ZNFs are some of these factors. Importantly, most of these regulators have only been studied in mouse models so far. A future understanding of the pathways operating in adult human tissues is essential for the development of innovative drugs. Targeted epigenetic modulation might prove a potent tool in the future to reactivate certain retrotransposons so that their nucleic acids and proteins could serve as natural PAMPs to signal danger to their host. Such drugs may be valuable to stimulate immunosurveillance in cancer patients in which immune activation pathways may be subdued or could be used in conjunction with standard vaccines in place of an adjuvant. Caution should be applied, however, to prevent unwanted effects of reactivated ERVs on the genome or transcriptome.

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Figure Legends

Figure 1: **Epigenetic factors repress retrotransposons in differentiated tissues, preventing transcription and sensing of non-self nucleic acids.** In differentiated cells, epigenetic silencing of retrotransposons is maintained by incompletely characterized mechanisms. Factors implicated in maintaining histone and DNA methylation at retrotransposons are shown. See text for details. Uncharacterized factors are depicted in blue to illustrate that this is an emerging area of research. The addition of 5-AZA-based drugs results in the generation of dsRNA from retrotransposons and potentially cDNA as well through reverse transcription, both of which lead to innate immune activation.