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Koala retrovirus and neoplasia: correlation and underlying mechanisms

Rachael Tarlinton[1] and Alex D Greenwood[2,3]

The koala retrovirus, KoRV, is one of the few models for understanding the health consequences of retroviral colonization of the germline. Such colonization events transition exogenous infectious retroviruses to Mendelian traits or endogenous retroviruses (ERVs). KoRV is currently in a transitional state from exogenous retrovirus to ERV, which in koalas (*Phascolarctos cinereus*) has been associated with strongly elevated levels of neoplasia. In this review, we describe what is currently known about the associations and underlying mechanisms of KoRV-induced neoplasia.

Addresses
[1] School of Veterinary Medicine and Science, University of Nottingham, Nottingham, UK
[2] Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
[3] School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Corresponding author: Greenwood, Alex D (greenwood@izw-berlin.de),

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Introduction

The koala retrovirus, KoRV, is unusual among known retroviruses in that, starting ca. 50 000 years ago, as opposed to millions of years ago for most known viruses, it has been in the process of colonizing the genome of its host, the koala (*Phascolarctos cinereus*) [1]. As a consequence of the recent germline colonization process, unlike most endogenous retroviruses (ERVs), KoRV is not fixed in the genome of koalas. This means that no KoRV proviral integrations are common to all koalas, with most being restricted to one or few individuals, specific populations, or geographic regions [1–3]. This contrasts with most known ERVs that colonized their host germlines millions of years ago and are, with few exceptions, fixed in all members of the host species [4]. For the koala host, the process of germline colonization has been associated with disease, primarily neoplasia in the form of extremely high incidences of leukemia and lymphoma, as well as severe Chlamydiosis [5–10].

Phylogenetic trees of endogenous and exogenous retroviruses demonstrate a long history of retroviral association with hosts, with the infectious exogenous viruses often becoming extinct. There are a small number of known groups of actively infectious retroviruses still causing horizontally transferrable disease (usually hematopoietic neoplasia) [11,12]. KoRV falls into the murine leukemia virus (MuLV) group of the gammaretrovirus genus; this group of related viruses includes most of the currently extant gammaretroviruses, including MuLV and feline leukemia virus (FeLV) [13]. FeLV has many biological parallels with KoRV and provides a useful pathology exemplar for retroviral disease. Like KoRV, FeLV has both endogenous and exogenous forms [14]. The endogenous form is found only in domestic felids and closely related species and is a relatively new entrant to the genome at less than 6 million years [15]. It is not fixed in the domestic cat genome with between 6 and 128 copies per individual [16]. It also has a horizontally transmitted exogenous version that causes leukemia and lymphoma in domestic cats by insertional mutagenesis in hematopoietic cells [17]. A variety of other disease outcomes are also induced by FeLV including immunosuppression, neuropathies, and reproductive failure [17]. The infectious version of FeLV also demonstrates repeated spillover from domestic cats into other felid species such as lynx and panthers [18,19]. Some cats clear infection, some suppress it to low levels, and some go on to develop uncontrolled viral replication and die from leukemia [17]. Vaccines against FeLV represent one successful example of a retroviral vaccine with a variety of inactivated, subunit, and recombinant vaccines controlling the disease in domestic cats [20]. FeLV also displays a propensity to mutation and recombination, altering host receptor usage and pathogenesis. The infectious variant (FeLV-A) that uses the host receptor THTR1 (thiamine transporter 1) recombines with its endogenous cousin resulting in a swap of envelope gene, *env*, receptor domains. These recombinants are known as FeLV-B and display enhanced pathogenesis and alternate host receptor usage (sodium-dependent phosphate transporter 1, PiT-1). Until recently, it was assumed that FeLV-B variants always arose within infections within individual cats and were not transmissible; however, recent carefully performed molecular phylogenetic studies have demonstrated that

FeLV-B variants can be transmitted without A variants [14]. There are also a number of other variants (C, E, and T) that arise from mutation of the FeLV A *env* gene and one variant (D) that arises from recombination with older ERVs in the cat genome (ERV-DC), as well as variants that have acquired cellular oncogenes [17].

The prototype gammaretrovirus is MuLV, with extensive literature on both endogenous and exogenous variants of MuLV and how these viruses trigger neoplasia. MuLV is another recent entrant into its murid hosts, with variants found throughout modern 'house mouse' *Mus musculus (musculus, domesticus, and castanaeus*) subspecies, breeds, and variants. *M. musculus* is thought to have originated in India between 0.5 and 1 million years ago, with their retroviruses evolving and spreading along with the mice themselves through human trade and shipping routes. The market for 'fancy' mouse breeding over several hundred years then led to the creation of the modern inbred laboratory mouse strains used as the backbone of biomedical research [21]. These mouse stains and their more outbred recent ancestors have a very variable complement of copies of MuLV between different strains, with MuLV insertion-induced genetic variation being responsible for a substantial portion of the total variation between strains [22]. There are also circulating exogenous infectious versions of MuLV, and the interactions between the two include blockade of infectious virus by defective variants [23], reactivation and new integrations of endogenous MuLV by pseudotyping with infectious MuLV [24], expression and recombination of nonfunctional MuLV alleles to form new infectious viruses [25], recombination between endogenous and exogenous MuLV to form new infectious virus variants [26], and the acquiring of cellular oncogenes in place of viral genes in infectious variants [27]. Indeed, this propensity of MuLV to package integrate and express nonviral sequences has led to an entire biotechnology field of MuLV-derived packaging vectors and viral pseudotypes used for gene expression and delivery in research and clinical applications [28]. Retroviruses including MuLV were also integral to the development of the concept of an oncogene, with the discovery of cellular genes in MuLV variants that induced rapid onset of cancer in infected mice. One of the most common (and still used) methods of oncogene discovery is by transformation of cell lines (or study of spontaneous tumors in infected mice) with MuLV and sequencing for enrichment of MuLV insertion sites in or near host genes in transformed cell lines [29].

The link between KoRV and neoplasia has been hypothesized since its discovery in koala tumor tissues [30,31]. Some of the earliest work on KoRV demonstrated a link between KoRV titer and neoplasia in koalas [5] that has been reproducible in multiple studies [9,32–34]. In addition, the closest relatives of KoRV, the various strains of gibbon ape leukemia virus (GALV), are oncogenic [13]. A GALV outbreak in the SEATO facility in Thailand led to high mortality with leukemia and lymphoma in the captive gibbon population. A GALV spillover to a captive woolly monkey co-housed with a gibbon identified the woolly monkey virus (WMV). GALV likely represents the infection of gibbons as a result of their handling in the SEATO facility and does not represent a virus of primates as it has never been isolated from gibbons outside the SEATO facility or animals not associated with the facility [13]. It is now clear that at least WMV and related strains are found in rodents and bats from Papua New Guinea and Australia [35–39], with Australian flying fox viruses almost certainly not endogenized and recently associated with lymphoma in gray-headed flying foxes (*Pteropus poliocephalus*) [40]. The close evolutionary relationship of KoRV and the GALVs, including their infection biology in cell culture, suggests oncogenic potential *in vivo* [41,42]. The first indication that exogenous KoRV variants exist and could be more strongly associated with neoplasia than the most widespread variant KoRV-A was the discovery of KoRV-B [43,44]. This variant has mutations concentrated in the viral long terminal repeats (LTRs) and in the envelope Variable Region A domain. The *env* mutations result in KoRV-B using THTR1 as a receptor rather than PiT-1, which is the receptor used by KoRV-A and the GALVs [43], similar to the difference between FeLV-A and FeLV-B. In a pedigree of koalas from the San Diego Zoological Garden, KoRV-B was suggested to be exclusively exogenous and more strongly associated with neoplasia than KoRV-A [43].

Since these initial discoveries, the field has progressed in large part by employing high throughput sequencing approaches. The availability of two high-quality koala reference genomes has also facilitated molecular work on KoRV [45,46]. Recent work has revealed a complex dynamic of KoRV sequence variants and their expression [47–49]. While many questions remain regarding how KoRV expression results in neoplasia, some clear molecular mechanisms have been identified, and new links between novel KoRV variants and their expression in koalas have been discovered, which are reviewed here.

Koala retrovirus viral load and cancer

One of the earliest observations of KoRV related to cancer was that KoRV viral titer is higher in koalas with neoplasia than in clinically healthy animals [5]. Most of the early work took place before KoRV variants had been identified, so it only measured general *pol* gene expression differences without differentiating which KoRVs were being expressed.

Recent studies have expanded on the higher obsserved titre taking into account KoRV variants. Zheng et al. demonstrated that non-KoRV-A (KoRV-B to KoRV-J)-associated viral loads were most strongly associated with leukemia and lymphoma, though KoRV-A was associated as well [32]. It is difficult to untangle the association between total viral load and viral diversity; however, several studies report a strong association between viral subtype diversity and increased viral load [49–51]. This is to be expected in a retroviral infection where increased viral replication gives rise to the opportunity for larger numbers of viral mutations to accumulate among the viral quasi-species. Antiviral treatment of a leukemic individual did not have any effect on KoRV viral integration [32]. However, only one individual was tested, and it is unclear how koala metabolism affects the efficacy of antivirals.

Northern and southern koalas show strong differences in the number of KoRV integrations they contain in their germline, which is often used as evidence to support the north to south expansion of KoRV in koala populations [47,48,52]. Northern koalas in Queensland and New South Wales (NSW) also have far more observed KoRV variants than koalas in Victoria and South Australia (SA). In fact, screening of isolated populations of koalas in SA and nearby islands suggested that a large proportion of koalas were KoRV free [52,53]. However, recent results demonstrate that these KoRV-free koalas carry recombinant KoRVs (recKoRVs) containing KoRV sequences flanking an ancient marsupial retroelement called PhER (Phascolarctos Endogenous Retrovirus), which largely replaces all the gene functions with nonfunctional distantly related retroviral sequence [54,55]. Sarker et al. demonstrated that expression differences were pronounced for KoRV between northern and southern koala populations [9]. Both proviral and viral load were drastically reduced in southern animals compared to northern. Higher viral loads were statistically associated with neoplasia, whereas low viral loads were correlated with other diseases found most prominently in southern koalas. Notably, disease manifestation in koalas is quite different from northern koalas (particularly in captive populations), which have extremely elevated rates of neoplasia compared to other mammals but are far less prominent in southern koalas [6,9].

Of particular interest, KoRV-A, and in some cases KoRV-B, infection of human cells (HEK293) supports the association with koala neoplasia *in vivo* [42]. This study performed RNAseq (illumina mRNAseq, Edge R analysis) on infected and uninfected cells and identified 760 annotated transcripts that were differentially expressed (p value < 0.05) with > 2-fold change. Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis identified 87 differentially regulated GO terms, many of which were clearly linked to antiviral immune responses. Nine KEGG terms were identified: systemic lupus erythematosus, alcoholism, cytokine–cytokine receptor interaction, transcriptional misregulation in cancer, neuroactive ligand–receptor interaction, NOD-like receptor signaling pathway, influenza A, viral carcinogenesis, and herpes simplex infection. Known oncogenes identified as differentially regulated included CSF3R, MMP9, REL, and RAS family (RAB17, RAB38, TLX1) genes. A number of histone genes (HIST1H3B, HIST1H3D, HIST1H3E, HIST1H3H) known to be involved in cell cycle progression defects, and DNA damage were also differentially regulated. Infection of HEK293 cells with KoRV-A therefore recapitulates expression patterns associated with oncogenesis such as increased expression of oncogenes associated with viral carcinogenesis, transcriptional misregulation in cancer in addition to increased expression of immune-associated genes, and genes associated with lupus. This is consistent with the immune dysregulation that has been associated with KoRV and clinical Chlamydia in koalas [56–58].

Viral *env* variants, expression, and cancer

Studies of KoRV-B suggest that the link to increased neoplasia may be more complex than just the presence or absence of KoRV-B, as KoRV-B positivity is associated with total KoRV viral load [7,33,49], and there has been a subsequent explosion in the number of KoRV variants identified since the description of KoRV-B [47,48,51,59,60]. A study of wild southern koalas (Victoria) did not find KoRV-B in the southern koala population [10]. KoRV-A was also found at a much lower prevalence than in Queensland or NSW. There was no link to KoRV and neoplasia in Victoria, but there was an association with clinical Chlamydial disease, both of these diseases being far less prevalent in southern populations than in northern ones [9,10].

A study of KoRV-A and KoRV-B in 290 animals collected over 5 years in Queensland examined these animals for *env* gene variation, transmission, and association with disease (neoplasia and chlamydia) [7]. As expected, KoRV-A prevalence was 100%, but in contrast to Legione's result, which focused on southern koalas, the prevalence of KoRV-B was 28%. KoRV-B presence correlated significantly with clinically detectable chlamydial disease. There was also a significant correlation with neoplasia and KoRV-B infection with observed neoplasia in 1.7% of the sampled koalas. The authors also describe evidence for dam-to-joey transmission of KoRV-B from 13 KoRV-B-positive dams that had 13 positive KoRV-B joeys, whereas 12 KoRV-B-negative dams had 12 KoRV-B-negative joeys.

Overall, some studies, such as Xu et al. and Quigley et al. 2018 [7,43], suggest a link between KoRV-B and higher rates of neoplasia, but this is difficult to untangle from the association of KoRV-B positivity with higher total viral load

(and overall viral diversity) [49,50]. With the contribution of KoRV-A to cancer and the tremendous variation between populations in terms of diversity and expression of KoRV-A and its variants, it will likely be difficult to tease out any one variant's higher oncogenic potential unless a new variant arises that is dramatically more oncogenic. However, it is unlikely that KoRV-B presents a much greater risk for neoplasia than other KoRV variants.

Koala retrovirus integrations underlying neoplasia

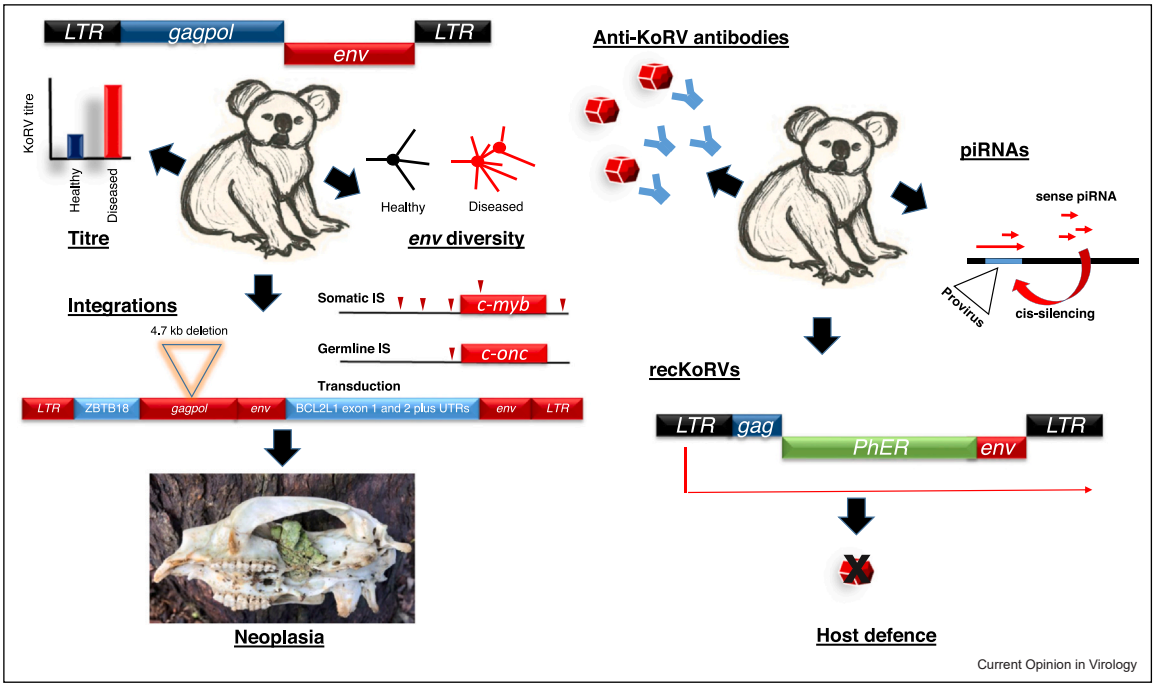
Expression changes and associations with variants are correlative but do not explain the underlying mechanisms of KoRV-derived neoplasia. McEwen et al. [2] investigated one underlying mechanism by identifying tumor-specific KoRV integrations comparing healthy to tumor tissue among animals. The authors identified three mechanisms underlying neoplasia that were almost exclusively associated with KoRV-A integrations. First, somatic mutations were found to accumulate in tumor tissue. The integrations did not occur randomly but were heavily enriched in genes associated with oncogenesis. These genes with integrations were found to have higher expression in tumor tissue than healthy tissue, suggesting that KoRV LTRs can promote overexpression of the genes in which they integrate. A second observation was the inheritance of integration sites (IS) that promoted neoplasia of the same kind. Three koalas from the same part of Australia shared IS in the 3′ UTR of *LSAMP*, a gene that is a candidate tumor suppressor in human osteosarcomas [61], and all developed osteosarcoma-related tumors during their lifetimes [2]. This suggests that inherited integrations can promote a lifetime risk of developing specific neoplasias. Another observation common to both the somatic integrations and the inherited IS in oncogenes was the observation of hot spots of integration where the same gene among and sometimes within the same animal had multiple KoRV integrations. In most cases, these were oncogenes, for example, *c-myb*. Thus, in both cases, not only were oncogenes more likely than non-oncogenes to have KoRV integrations, but they also experienced multiple KoRV integrations both among and within animals. In almost all cases, the observed integrations were KoRV-A sequences with rarer demonstration of recKoRV integrations. There was no evidence of extensive contribution of KoRV variants to this process. It should also be noted that only KoRV-A variants have been demonstrated to endogenize [62,63] and that it is not certain that variants other than A and B are actually fully replication competent and capable of completing the retroviral life cycle as other variants fail to passage in cell culture [42,43].

A final oncogenic mechanism observed was retroviral transduction. One of the first described retroviruses, Rous sarcoma virus, was found to have transduced the chicken host *src* gene, placing a modified *src* gene under control of the retrovirus, leading to misexpression of *src* and development of sarcomas in chickens, which were infectious [64]. In two koalas tested, McEwen et al. described the transduction of the BCL2L oncogenes by KoRV-A [2]. In one koala, the gene was disrupted, and the two koala transductions had somewhat different structures. This suggests that they may have occurred independently. In one koala, the BCL2L gene was entirely intact and replaced most of the retroviral genes with the exception of some residual *gag* and *env* sequences remaining. In the koala with the transduced BCL2L gene, expression of this oncogene was increased in tumor tissue by over 500-fold relative to koalas without the transduced oncogene. Whether the transduced retrovirus is infectious to other koalas remains unresolved.

Koala host responses to koala retrovirus

While not strictly anticancer mechanisms, koalas have a variety of molecular mechanisms that have evolved to limit KoRV expression, some KoRV specific and some more generally antiviral. One of the first potential defense mechanisms is the formation of recKoRVs [54,55]. These elements form when KoRV recombines with an ancient marsupial endogenous retroelement called Phascolarctos Endogenous Retrovirus, PhER. This is likely driven by microhomologies between the two viruses and may either occur during reverse transcription, as both KoRV and PhER are expressed or during integration of retroviral DNA. The resulting recKoRVs lack all or most retroviral genes replacing them with extensively mutated PhER sequences that are unable to produce functional retroviral proteins. In Queensland, recKoRVs make up a large proportion of the total KoRVs (10% or more) in a given individual [55]. At least 17 distinct recKoRVs have been identified and some such as recKoRV1 appear to have been generated independently multiple times in different koala populations. In KoRV 'negative' SA koalas, all appear to have a newly described recKoRV but no intact KoRV [54]. KoRV copies are often very low and likely never homozygous with a likely explanation being that SA koalas have lost KoRV by genetic drift and recKoRV has remained. While recKoRVs have also been associated with integration-driven oncogenesis [2], over time, the more recKoRVs come to represent the dominant KoRV subtype in koalas, the less neoplasia they will experience. In support of this, the southern koalas with little KoRV and only recKoRVs suffer much lower rates of neoplasia than their northern counterparts [9]. The mechanisms for this are not clear, but it does appear to be a feature of the age and functionality of retroelements in genomes [65] and may in the first instance be similar to the blockade of infectious variants of MuLV by nonfunctional endogenous counterparts [21]. Thus, the recombination-driven loss of function of KoRV may be brought about by PhER as a genomic defense mechanism.

Figure 1



Summary of correlates and involvement of KoRV in neoplasia and host anti-KoRV defense. In the left panel, the associated increased expression of KoRV and increased expression of variants is shown above. Below the three KoRV integration-associated molecular mechanisms underlying neoplasia are shown including somatic integrations in oncogenes (including multiple independent integrations in the same gene), germline integrations in oncogenes predisposing koalas to specific neoplasias, and transduction of oncogenes. An example of a koala with a tumor is shown in the picture below. In the right panel, the host defenses against KoRV are summarized including piRNAs, anti-KoRV antibodies, and recKoRVs. The drawn koala image was provided by Saba Mottaghinia and the koala neoplasia image by Amber Gillett.

Another defense against KoRV expression and hence a potential mitigator of neoplasia are Piwi-interacting RNAs (piRNAs), which are antisense RNAs that normally inhibit transposon expression in trans by generating antisense homologs of the transposons they suppress and sequestering the expressed RNA, thereby preventing transposition [66]. KoRV being an extremely young retrovirus of koalas does not induce a full piRNA pathway. During KoRV infection, sense strand piRNAs are generated from unspliced KoRV transcripts, which may halt replication in cis until antisense piRNAs are generated that can then suppress KoRV expression in trans [66]. The system is clearly not completely functional as full-length KoRV transcripts and genomes are produced despite this inhibition. However, suppression of KoRV, even if only moderate, would likely decrease the negative effects of expression compared to unrestricted KoRV expression.

The two preceding host response mechanisms are intracellular anti-KoRV systems. A likely extracellular defense against KoRV is provided by the immune system. The difficulty for koalas in the northern populations is that there are insufficient differences between endogenous and infectious KoRV variants to be distinguished by the immune system, unlike FeLV in cats, where a robust immune response to FeLV does develop [20]. Animals expressing KoRV antigens during fetal development will likely recognize KoRV as a 'self' antigen and not respond to it. This phenomenon of persistent infection due to fetal infection is well described in veterinary medicine [67], and this difference between southern populations lacking endogenous KoRV and northern koala populations born with it (and perhaps unable to control infection) has been postulated as one reason for the stark difference in disease incidence between the two groups [54]. Studies of native antibody levels and response to vaccination with KoRV Env proteins [68–74] have produced conflicting results as to whether northern koalas recognize KoRV. The first study of immune response to KoRV performed Western blot analysis using either cell-cultured KoRV lysate or recombinant rp15E or gp70 proteins. While positive control sera was positive, 13 koala serum samples were negative on Western blot [72]. Similar results were obtained by Joyce et al. [75] in which they used a clamp trimerization domain to maintain the KoRV Env structure. While positive controls were again positive, 8 captive and wild koala sera were negative. In contrast, Waugh et al. [71] and Olagoke et al. [68,69,76] reported strong KoRV antibody signals by enzyme-linked immunosorbent assay and viral neutralization assays in large cohorts of koalas. These contradictory results remain

unresolved. However, Denner [77] raises the possibility that the studies detecting KoRV immune response are actually measuring Glutathione S-transferases or bacterial proteins used in developing the KoRV proteins, and Joyce et al. [75] suggest, in addition, that by not using proteins that maintain native structure, the positive results may not represent response to KoRV. Currently, the issue is unresolved; however, it is likely that KoRV is ignored by the immune system and therefore able to proliferate both intra and extracellularly with little inhibition in Northern animals. Southern animals (not born with endogenous KoRV) likely do mount an immune response to it [68,69,76].

Similarly, susceptibility to cancer may also have an immune basis once transformation and neoplasia is initiated. A study of koala immunogenetic diversity including healthy and leukemic koalas demonstrated a statistically significant association between the Donor Bone Marrow*03 major histocompatibility complex Class II allele and the likelihood of developing cancer [78]. There are also a large number of other genetic differences between koala populations that could also play a role in disease pathogenesis [79]. While not a direct link to causation, it does suggest that resistance and defense against KoRV-initiated neoplasia are mediated by the koala immune system.

Future perspectives

The over-riding conservation and animal welfare concern with KoRV is whether the disease impacts of the virus can be reduced in both wild and managed populations. The argument for KoRV as the causal agent of cancer in koalas is as close to proven as it can be without an experimental infection, a difficult thing to justify in a vulnerable wild species with strong epidemiological evidence linking KoRV load and neoplasia [5,9,53], cell culture evidence of activation of cancer pathways [42], and a demonstrated mechanism of insertional mutagenesis in, near, or co-opting known oncogenes [2]. Vaccination is one management tool being pursued for the control of KoRV [80]. Selective breeding to eliminate or reduce the incidence of KoRV IS associated with oncogenes is another potential strategy, with the sequencing of complete zoo population pedigrees a next step in exploring the feasibility of this approach. Much work remains to be done to examine the differential KoRV profiles, immune responses, and KoRV restriction mechanisms between the northern and southern koala populations with implications both for disease impacts in the koala population and the fundamentals of genome evolution and variation driven by retroviruses. Figure 1.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Ishida Y, Zhao K, Greenwood AD, Roca AL: **Proliferation of endogenous retroviruses in the early stages of a host germ line invasion**. *Mol Biol Evol* 2015, **32**:109-120, https://doi.org/10.1093/molbev/msu275.
This is the first study to characterize integration sites of the KoRV and estimate the date of germline colonization.

2. •• McEwen G, *et al.*: **Retroviral integrations contribute to elevated host cancer rates during germline invasion**. *Nat Commun* 2021, **12**:1316.
This study examined the differences in KoRV IS profiles between healthy and tumor koala tissue samples. The authors demonstrated that KoRV somatic integrations accumulate preferentially in oncogenes influencing their expression in tumor tissue.

3. Tsangaras K, *et al.*: **Hybridization capture reveals evolution and conservation across the entire Koala retrovirus genome**. *PLoS One* 2014, **9**:e95633, https://doi.org/10.1371/journal.pone.0095633

4. • Denner J: In *Retroviruses*. Edited by Kurth R, Bannert N. Caister Academic Press; 2010:35-70.
This is a solid basic primer on how endogenous and exogenous retroviruses function and overlap.

5. • Tarlinton R, Meers J, Hanger J, Young P: **Real-time reverse transcriptase PCR for the endogenous koala retrovirus reveals an association between plasma viral load and neoplastic disease in koalas**. *J Gen Virol* 2005, **86**:783-787, https://doi.org/10.1099/vir.0.80547-0.
The authors demonstrate an association between extracellular viral load of the KoRV and the development of neoplasia in koalas.

6. •• Fabijan J, *et al.*: **Pathological findings in koala retrovirus-positive koalas (*Phascolarctos cinereus*) from Northern and Southern Australia**. *J Comp Pathol* 2020, **176**:50-66, https://doi.org/10.1016/j.jcpa.2020.02.003.
This is one of the few large pathology studies to directly compare northern and southern populations of koalas.

7. • Quigley BL, Ong VA, Hanger J, Timms P: **Molecular dynamics and mode of transmission of koala retrovirus as it invades and spreads through a wild Queensland koala population**. *J Virol* 2018, **92**, https://doi.org/10.1128/jvi.01871-17.
The authors examined KoRV envelope subtypes in a wild koala population from Queensland. They suggest that KoRV-B is associated with Chlamydia and neoplasia and that transmission of KoRV-B is largely from mother to Joey.

8. Waugh CA, *et al.*: **Infection with koala retrovirus subgroup B (KoRV-B), but not KoRV-A, is associated with chlamydial disease in free-ranging koalas (*Phascolarctos cinereus*)**. *Sci Rep* 2017, **7**:134, https://doi.org/10.1038/s41598-017-00137-4

9. • Sarker N, *et al.*: **Koala retrovirus viral load and disease burden in distinct northern and southern koala populations**. *Sci Rep* 2020, **10**:263, https://doi.org/10.1038/s41598-019-56546-0.
The authors demonstrate that viral loads and proviral copy are high in northern koala populations for the KoRV and both proviral and viral loads are low in southern koala populations. In both populations, there was a significant association between viral load and neoplasia.

10. • Legione AR, *et al.*: **Koala retrovirus genotyping analyses reveal a low prevalence of KoRV-A in Victorian koalas and an**

association with clinical disease. *J Med Microbiol* 2017, **66**:236-244, https://doi.org/10.1099/jmm.0.000416.
The authors clearly show that KoRV-A prevalence is much lower in southern koalas than in northern and KoRV-B was absent. The presence of KoRV provirus was significantly correlated with koala disease.

11. Hayward A, Cornwallis CK, Jern P: **Pan-vertebrate comparative genomics unmasks retrovirus macroevolution**. *Proc Natl Acad Sci USA* 2015, **112**:464-469, https://doi.org/10.1073/pnas.1414980112

12. Hayward A, Grabherr M, Jern P: **Broad-scale phylogenomics provides insights into retrovirus-host evolution**. *Proc Natl Acad Sci USA* 2013, **110**:20146-20151, https://doi.org/10.1073/pnas.1315419110

13. Brown K, Tarlinton R: **Is gibbon ape leukaemia virus still a threat?** *Mammal Rev* 2017, **47**:53-61.

14. Erbeck K, *et al*.: **Feline leukemia virus (FeLV) endogenous and exogenous recombination events result in multiple FeLV-B subtypes during natural infection**. *J Virol* 2021, **95**:e0035321, https://doi.org/10.1128/jvi.00353-21

15. Benveniste RE, Todaro GJ: **Segregation of RD-114 AND FeL-V-related sequences in crosses between domestic cat and leopard cat**. *Nature* 1975, **257**:506-508, https://doi.org/10.1038/257506a0

16. Tandon R, *et al*.: **Copy number polymorphism of endogenous feline leukemia virus-like sequences**. *Mol Cell Probes* 2007, **21**:257-266, https://doi.org/10.1016/j.mcp.2007.01.003

17. Hartmann K, Hofmann-Lehmann R: **What's new in feline leukemia virus infection**. *Vet Clin North Am Small Anim Pract* 2020, **50**:1013-1036, https://doi.org/10.1016/j.cvsm.2020.05.006

18. Chiu ES, *et al*.: **Multiple introductions of domestic cat feline leukemia virus in endangered Florida panthers**. *Emerg Infect Dis* 2019, **25**:92-101, https://doi.org/10.3201/eid2501.181347

19. Meli ML, *et al*.: **Feline leukemia virus infection: a threat for the survival of the critically endangered Iberian lynx (Lynx pardinus)**. *Vet Immunol Immunopathol* 2010, **134**:61-67, https://doi.org/10.1016/j.vetimm.2009.10.010

20. Little S, *et al*.: **2020 AAFP Feline Retrovirus Testing and Management Guidelines**. *J Feline Med Surg* 2020, **22**:5-30, https://doi.org/10.1177/1098612x19895940

21. Kozak CA: **Origins of the endogenous and infectious laboratory mouse gammaretroviruses**. *Viruses* 2014, **7**:1-26, https://doi.org/10.3390/v7010001

22. Nellaker C, *et al*.: **The genomic landscape shaped by selection on transposable elements across 18 mouse strains**. *Genome Biol* 2012, **13**:R45.

23. Nethe M, Berkhout B, van der Kuyl AC: **Retroviral superinfection resistance**. *Retrovirology* 2005, **2**:52, https://doi.org/10.1186/1742-4690-2-52

24. • Evans LH, *et al*.: **Mobilization of endogenous retroviruses in mice after infection with an exogenous retrovirus**. *J Virol* 2009, **83**:2429-2435.
Description of the mechanism by which exogenous infection reactivates endogenous retroviruses in mice.

25. Stoye JP, Moroni C, Coffin JM: **Virological events leading to spontaneous AKR thymomas**. *J Virol* 1991, **65**:1273-1285.

26. Boi S, *et al*.: **Endogenous retroviruses mobilized during friend murine leukemia virus infection**. *Virology* 2016, **499**:136-143, https://doi.org/10.1016/j.virol.2016.07.009

27. Fan H: **Murine leukemia viruses**. Encyclopaedia of Virology. Elsevier; 1999:995-1001.

28. Maetzig T, Galla M, Baum C, Schambach A: **Gammaretroviral vectors: biology, technology and application**. *Viruses* 2011, **3**:677-713, https://doi.org/10.3390/v3060677

29. Haeri M, *et al*.: **Insertional activation of myb by F-MuLV in SCID mice induces myeloid leukemia**. *Int J Oncol* 2013, **43**:169-176, https://doi.org/10.3892/ijo.2013.1943

30. • Canfield PJ, Sabine JM, Love DN: **Virus particles associated with leukaemia in a koala**. *Aust Vet J* 1988, **65**:327-328, https://doi.org/10.1111/j.1751-0813.1988.tb14518.x.
The authors present the first data that associated the high prevalence of leukemia in koalas with a viral pathogen.

31. •• Hanger JJ, Bromham LD, McKee JJ, O'Brien TM, Robinson WF: **The nucleotide sequence of koala (*Phascolarctos cinereus*) retrovirus: a novel type C endogenous virus related to gibbon ape leukemia virus**. *J Virol* 2000, **74**:4264-4272, https://doi.org/10.1128/jvi.74.9.4264-4272.2000.
The authors presented the first sequence of the KoRV.

32. • Zheng H, *et al*.: **Koala retrovirus diversity, transmissibility, and disease associations**. *Retrovirology* 2020, **17**:34, https://doi.org/10.1186/s12977-020-00541-1.
A large cohort of captive and wild koalas were examined for KoRV variant provirus and plasma titer demonstrating an association of non-KoRV-A variants and KoRV-A with neoplasia. Exclusive dam to joey transmission was not supported through what appeared to be the dominant transmission mechanism. Anti-retroviral treatment of a leukemic koala did not alter KoRV expression which was surprising.

33. Hashem MA, *et al*.: **Koala retrovirus (KoRV) subtypes and their impact on captive koala (*Phascolarctos cinereus*) health**. *Arch Virol* 2021, **166**:1893-1901, https://doi.org/10.1007/s00705-021-05078-y

34. Stephenson T, *et al*.: **Molecular diagnosis of koala retrovirus (KoRV) in South Australian koalas (*Phascolarctos cinereus*)**. *Animals* 2021, **11**, https://doi.org/10.3390/ani11051477

35. • Simmons G, Clarke D, McKee J, Young P, Meers J: **Discovery of a novel retrovirus sequence in an Australian native rodent (*Melomys burtoni*): a putative link between gibbon ape leukemia virus and koala retrovirus**. *PLoS One* 2014, **9**:e106954, https://doi.org/10.1371/journal.pone.0106954.
The authors describe a partial sequence of a WMV-like endogenous retroviral sequence identified in Australian populations of *Melomys burtoni*.

36. • Alfano N, *et al*.: **Endogenous gibbon ape leukemia virus identified in a rodent (*Melomys burtoni* subsp.) from Wallacea (Indonesia)**. *J Virol* 2016, **90**:8169-8180, https://doi.org/10.1128/jvi.00723-16.
The authors describe the full-length endogenous woolly monkey-like retrovirus in *Melomys burtoni* from Indonesia. The full provirus and flanking sequence were described and the endogenized copy lacked complete genes suggesting it is unable to form infectious particles.

37. • McMichael L, *et al*.: **A novel Australian flying-fox retrovirus shares an evolutionary ancestor with Koala, Gibbon and Melomys gamma-retroviruses**. *Virus Genes* 2019, **55**:421-424, https://doi.org/10.1007/s11262-019-01653-3.
The first KoRV and gibbon ape leukemia-like virus identified in a bat from Australia is described by the authors.

38. • Hayward JA, *et al*.: **Infectious KoRV-related retroviruses circulating in Australian bats**. *Proc Natl Acad Sci USA* 2020, **117**:9529-9536, https://doi.org/10.1073/pnas.1915400117.
Several gibbon ape leukemia-like retroviruses are described from Australian bats, and partial fragments of related viruses are described from Chinese bats. The study suggests that both rodents and bats harbor KoRV-like viruses.

39. • Mottaghinia S, *et al*.: **A recent gibbon ape leukemia virus germline integration in a rodent from New Guinea**. *Proc Natl Acad Sci USA* 2024, **21**:e2220392121.
The authors describe a fully intact endogenous woolly monkey retrovirus in *Melomys leucogaster* from New Guinea. The authors demonstrate that the endogenized virus has remained infectious and is at the earliest stage of genome colonization in its host making it an even younger model than KoRV.

40. Van Brussel K, *et al*.: **Gammaretroviruses, novel viruses and pathogenic bacteria in Australian bats with neurological signs, pneumonia and skin lesions**. *Virology* 2023, **586**:43-55, https://doi.org/10.1016/j.virol.2023.07.011

41. • Oliveira NM, Farrell KB, Eiden MV: **In vitro characterization of a koala retrovirus**. *J Virol* 2006, **80**:3104-3107, https://doi.org/10.1128/jvi.80.6.3104-3107.2006.
Using viral constructs and cell culture models the authors demonstrate the major changes between GALV and KoRV, which make KoRV less virulent.

42. • Sarker N, *et al*.: **Novel insights into viral infection and oncogenesis from koala retrovirus (KoRV) infection of**

HEK293T cells. *Gene* 2020, **733**:144366, https://doi.org/10.1016/j.gene.2020.144366.
The authors demonstrate which genes alter expression upon infection of human cell lines with KoRV.

43. •• Xu W, *et al*.: **An exogenous retrovirus isolated from koalas with malignant neoplasias in a US zoo**. *Proc Natl Acad Sci USA* 2013, **110**:11547-11552, https://doi.org/10.1073/pnas.1304704110.
The study is the first description of KoRV-B and suggests it is more virulent than KoRV-B.

44. Shojima T, *et al*.: **Identification of a novel subgroup of Koala retrovirus from Koalas in Japanese zoos**. *J Virol* 2013, **87**:9943-9948, https://doi.org/10.1128/jvi.01385-13

45. Blanchard AM, *et al*.: **Genome reference assembly for bottlenecked Southern Australian koalas**. *Genome Biol Evol* 2023, **15**, https://doi.org/10.1093/gbe/evac176

46. Johnson RN, *et al*.: **Adaptation and conservation insights from the koala genome**. *Nat Genet* 2018, **50**:1102-1111, https://doi.org/10.1038/s41588-018-0153-5

47. • Blyton MDJ, Young PR, Moore BD, Chappell KJ: **Geographic patterns of koala retrovirus genetic diversity, endogenization, and subtype distributions**. *Proc Natl Acad Sci USA* 2022, **119**:e2122680119, https://doi.org/10.1073/pnas.2122680119.
The authors describe the characterization of KoRV variants across much of the koala range, demonstrating a hard barrier to subtype transmission between the NSW – Victoria border. All variants of KoRV except for KoRV-A were absent in Victoria.

48. Quigley BL, Wedrowicz F, Hogan F, Timms P: **Phylogenetic and geographical analysis of a retrovirus during the early stages of endogenous adaptation and exogenous spread in a new host**. *Mol Ecol* 2021, **30**:2626-2640, https://doi.org/10.1111/mec.15735

49. • Sarker N, *et al*.: **Genetic diversity of Koala retrovirus env gene subtypes: insights into northern and southern koala populations**. *J Gen Virol* 2019, **100**:1328-1339, https://doi.org/10.1099/jgv.0.001304.
The authors demonstrate the distinct differences in subtype diversity and expression of KoRV subtypes in northern versus southern koala populations. Southern populations exhibit far less diversity and expression of KoRV.

50. • Quigley BL, *et al*.: **Changes in endogenous and exogenous koala retrovirus subtype expression over time reflect koala health outcomes**. *J Virol* 2019, **93**, https://doi.org/10.1128/jvi.00849-19.
Wild cohorts of koalas were examined for KoRV variant expression over time. Animals that remained healthy tended to express KoRV-D, while those that went on to develop Chlamydia infections expressed multiple subtypes.

51. • Blyton MDJ, Pyne M, Young P, Chappell K: **Koala retrovirus load and non-A subtypes are associated with secondary disease among wild northern koalas**. *PLoS Pathog* 2022, **18**:e1010513, https://doi.org/10.1371/journal.ppat.1010513.
Wild koalas expressing KoRV-D appeared to develop Chlamydia more frequently than those that did not and KoRV-B appeared to play no role in the development of disease. The best predictor of disease development was the overall KoRV load.

52. Simmons GS, *et al*.: **Prevalence of koala retrovirus in geographically diverse populations in Australia**. *Aust Vet J* 2012, **90**:404-409, https://doi.org/10.1111/j.1751-0813.2012.00964.x

53. •• Tarlinton RE, Meers J, Young P: **Retroviral invasion of the koala genome**. *Nature* 2006, **422**:79-81.
The authors for the first time demonstrated that KoRV is a virus still colonizing the koala genome in real-time.

54. •• Tarlinton RE, *et al*.: **Differential and defective transcription of koala retrovirus indicates the complexity of host and virus evolution**. *J Gen Virol* 2022, **103**, https://doi.org/10.1099/jgv.0.001749.
The authors demonstrated that KoRV-negative southern koalas in fact contain a recombinant KoRV lacking the genes that are generally used to diagnose the presence or absence of KoRV. However, they lack intact KoRV for the most part.

55. • Lober U, *et al*.: **Degradation and remobilization of endogenous retroviruses by recombination during the earliest stages of a germ-line invasion**. *Proc Natl Acad Sci USA* 2018, **115**:8609-8614, https://doi.org/10.1073/pnas.1807598115.
The authors identified recombinant versions of KoRV (recKoRVs) which replace much of the KoRV genome with much older marsupial retroviral sequences. These recKoRVs are diverse, re-occuring, and expanding in the genome of koalas.

56. Maher IE, Patterson J, Curnick M, Devlin J, Higgins DP: **Altered immune parameters associated with koala retrovirus (KoRV) and chlamydial infection in free ranging Victorian koalas (*Phascolarctos cinereus*)**. *Sci Rep* 2019, **9**:11170, https://doi.org/10.1038/s41598-019-47666-8

57. Maher IE, Higgins DP: **Altered immune cytokine expression associated with KoRV B infection and season in captive koalas**. *PLoS One* 2016, **11**:e0163780, https://doi.org/10.1371/journal.pone.0163780

58. Fernandez CM, *et al*.: **A novel multi-variate immunological approach, reveals immune variation associated with environmental conditions, and co-infection in the koala (*Phascolarctos cinereus*)**. *Sci Rep* 2024, **14**:7260, https://doi.org/10.1038/s41598-024-57792-7

59. • Chappell KJ, *et al*.: **Phylogenetic diversity of koala retrovirus within a wild koala population**. *J Virol* 2017, **91**, https://doi.org/10.1128/jvi.01820-16.
The study represents one of the first descriptions of the nearly full diversity of KoRV variants in wild Australian koalas based on deep sequencing.

60. Joyce BA, Blyton MDJ, Johnston SD, Young PR, Chappell KJ: **Koala retrovirus genetic diversity and transmission dynamics within captive koala populations**. *Proc Natl Acad Sci USA* 2021, **118**, https://doi.org/10.1073/pnas.2024021118

61. Kresse SH, *et al*.: **LSAMP, a novel candidate tumor suppressor gene in human osteosarcomas, identified by array comparative genomic hybridization**. *Genes Chromosomes Cancer* 2009, **48**:679-693, https://doi.org/10.1002/gcc.20675

62. • Avila-Arcos MC, *et al*.: **One hundred twenty years of koala retrovirus evolution determined from museum skins**. *Mol Biol Evol* 2013, **30**:299-304, https://doi.org/10.1093/molbev/mss223.
The authors present the first study of DNA from koala museum skins showing that KoRV changed very little over the last 120 years suggesting little virus–host adaptation.

63. • Hobbs M, *et al*.: **Long-read genome sequence assembly provides insight into ongoing retroviral invasion of the koala germline**. *Sci Rep* 2017, **7**:15838, https://doi.org/10.1038/s41598-017-16171-1.
The authors describe full and near full-length KoRV sequences from a koala including the first description of recKoRV1.

64. Rubin H: **The early history of tumor virology: Rous, RIF, and RAV**. *Proc Natl Acad Sci USA* 2011, **108**:14389-14396, https://doi.org/10.1073/pnas.1108655108

65. Kassiotis G: **The immunological conundrum of endogenous retroelements**. *Annu Rev Immunol* 2023, **41**:99-125, https://doi.org/10.1146/annurev-immunol-101721-033341

66. Kassiotis G: **The piRNA response to retroviral invasion of the koala genome**. *Cell* 2019, **179**:632-643, https://doi.org/10.1016/j.cell.2019.09.002

67. Lanyon SR, Hill FI, Reichel MP, Brownlie J: **Bovine viral diarrhoea: pathogenesis and diagnosis**. *Vet J* 2014, **199**:201-209, https://doi.org/10.1016/j.tvjl.2013.07.024

68. Olagoke O, *et al*.: **Induction of neutralizing antibody response against koala retrovirus (KoRV) and reduction in viral load in koalas following vaccination with recombinant KoRV envelope protein**. *NPJ Vaccines* 2018, **3**:30, https://doi.org/10.1038/s41541-018-0066-4

69. Olagoke O, Quigley BL, Eiden MV, Timms P: **Antibody response against koala retrovirus (KoRV) in koalas harboring KoRV-A in the presence or absence of KoRV-B**. *Sci Rep* 2019, **9**:12416, https://doi.org/10.1038/s41598-019-48880-0

70. Olagoke O, Quigley BL, Hemmatzadeh F, Tzipori G, Timms P: **Therapeutic vaccination of koalas harbouring endogenous koala retrovirus (KoRV) improves antibody responses and reduces circulating viral load**. *NPJ Vaccines* 2020, **5**:60, https://doi.org/10.1038/s41541-020-0210-9

71. Waugh C, Gillett A, Polkinghorne A, Timms P: **Serum antibody response to koala retrovirus antigens varies in free-ranging**

koalas (*Phascolarctos cinereus*) in Australia: implications for vaccine design. *J Wildl Dis* 2016, **52**:422-425, https://doi.org/10.7589/2015-09-257

72. Fiebig U, *et al*.: **Induction of neutralizing antibodies specific for the envelope proteins of the koala retrovirus by immunization with recombinant proteins or with DNA**. *Virol J* 2015, **12**:68, https://doi.org/10.1186/s12985-015-0296-2

73. Fiebig U, Hartmann MG, Bannert N, Kurth R, Denner J: **Transspecies transmission of the endogenous koala retrovirus**. *J Virol* 2006, **80**:5651-5654, https://doi.org/10.1128/jvi.02597-05

74. Fiebig U, Keller M, Moller A, Timms P, Denner J: **Lack of antiviral antibody response in koalas infected with koala retroviruses (KoRV)**. *Virus Res* 2015, **198**:30-34, https://doi.org/10.1016/j.virusres.2015.01.002

75. Joyce BA, Watterson D, Isaacs A, Chappell KJ: **Concerns regarding vaccination as a management strategy against koala retrovirus**. *bioRxiv* 2021, 2021-05.

76. Olagoke O, Quigley BL, Hemmatzadeh F, Tzipori G, Timms P: **Therapeutic vaccination of koalas harbouring endogenous koala retrovirus (KoRV) improves antibody responses and reduces circulating viral load**. *NPJ Vaccines* 2020, **5**:60, https://doi.org/10.1038/s41541-020-0210-9

77. Denner J: **Vaccination against the koala retrovirus (KoRV): problems and strategies**. *Animals* 2021, **11**, https://doi.org/10.3390/ani11123555.
• Review paper of vaccination for KoRV in Koalas.

78. Robbins A, Hanger J, Jelocnik M, Quigley BL, Timms P: **Koala immunogenetics and chlamydial strain type are more directly involved in chlamydial disease progression in koalas from two south east Queensland koala populations than koala retrovirus subtypes**. *Sci Rep* 2020, **10**:15013, https://doi.org/10.1038/s41598-020-72050-2.
• An epidemiological study of KoRV and Chlamydial co-infections and immune gene genetics in northern koalas.

79. Tarlinton R, *et al*.: **Transcriptomic and genomic variants between koala populations reveals underlying genetic components to disorders in a bottlenecked population**. *Conserv Genet* 2021, **22**:329-340, https://doi.org/10.1007/s10592-021-01340-7.
• An mRNAseq study of Non-KoRV genetic differences between northern and southern animals, describes potential candidate gene underlying oxalate nephrosis in South Australian animals.

80. Quigley BL, Timms P: **Helping koalas battle disease — recent advances in chlamydia and koala retrovirus (KoRV) disease understanding and treatment in koalas**. *FEMS Microbiol Rev* 2020, **44**:583-605, https://doi.org/10.1093/femsre/fuaa024