The Koala and its Retroviruses:
Implications for Sustainability and Survival

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Potential Role of Human Restriction Factors in Inhibiting the Emergence of Koala Retrovirus (KoRV) as a Zoonotic Agent

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Abstract. The findings of an exogenous koala retrovirus (KoRV) associated with neoplastic diseases in koalas *(Phascolarctos cinereus)* brought up the concerns of infection by koala retroviruses in humans, especially koala handlers. As simple retroviruses, koala retroviruses lack the regulatory genes to counter restriction activities by human restriction factors in viral replication. Koala retroviruses belong to gammaretroviruses. Previous studies of susceptibility of murine leukemia virus and a lab contaminant retrovirus, gammaretroviral xenotropic MLV-related virus, to human restriction factors disprove the possibility of gammaretrovirus as a human pathogen. There is no evidence that the koala retrovirus can infect and replicate in human peripheral blood mononuclear cells, which is consistent with the resistant role of human restriction factors against gammaretroviruses.

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Retroviruses have existed and co-evolved with eukaryotic cells for millions of years. According to the genome organization, retroviruses can be divided into two broad groups, "simple" and "complex" viruses. Simple retroviruses contain only *gag, pol,* and *env* genes. Gammaretroviruses, including murine leukemia virus (MLV), feline leukemia virus (FeLV), gibbon ape leukemia virus (GALV), and koala retrovirus (KoRV) are simple viruses. Complex viruses contain regulatory/accessory genes in addition to their functional genes. A well-known example of a complex retrovirus is human immunodeficiency virus-1 (HIV-1) that contains two regulatory and four accessory genes; the latter appear to be dedicated to evade host defenses.

Mammalian cells have developed various innate self-defense mechanisms during the long battle to defend against infection by retroviruses. Among these anti-viral mechanisms three major classes of human restriction factors that block or restrict retroviral replication at different stages of life cycle have been described in detail through the studies of ecotropic MLV and HIV-1, including the APOBEC3 family of DNA cytidine deaminases, tripartite motif protein 5-alpha (TRIM5α), and tetherin (Malim, 2009; Wolf & Goff, 2008).

Three potent human restriction factors

The APOBEC3 restriction system comprises a family of polynucleotide cytidine deaminases. APOBEC3 proteins can be efficiently packaged into retroviral particles and inhibit replication by deaminating cytosine residues converting them to uracil during the first step of reverse transcription-the synthesis of minus strand DNA, which in turn, results in the guanine to adenine transition mutations in plus strand

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DNA in the infected cells (Malim, 2009; Wolf & Goff, 2008). The cytidine deaminase activity of human APOBEC3G and 3F can be neutralized by viral infectivity factor (Vif), an accessory protein of HIV, which can interact with APOBEC3 proteins and induce cellular proteasomal pathway to degrade these proteins (Marin *et al*., 2003; Mehle *et al*., 2004; Sheehy *et al*., 2003).

TRIM5α is a restriction factor first identified during the studies of the resistance to HIV-1 infection in old world monkeys (Stremlau *et al*., 2004), It belongs to the tripartite motif family, and contains a variable C-terminal SPRY or B30.2 domain that recognizes the capsid protein of an incoming retrovirus and determines the ability of TRIM5α to restrict specific retroviruses (Nisole *et al*., 2005; Perez-Caballero *et al*., 2005). TRIM5α inhibits infection subsequent to retroviral entry and delivery of the viral core into cytoplasm. It affects various retroviral core components and is proposed to cause premature disassembly and/or degradation of the reverse transcription complex, or block the nuclear translocation of the preintegration complex (Kutluay *et al*., 2013). The molecular mechanism of TRIM5α-mediated restriction is not fully understood. TRIM5α demonstrates some specificity in its restrictive capabilities. Human TRIM5α strongly inhibits MLV-N tropic and Equine Infectious Anemia Virus, but not MLV-B tropic, HIV-1 or Simian Immunodeficiency Retrovirus of Macaques (Keckesova *et al*., 2004; Perron *et al*., 2004).

Tetherin (previously known as HM1.24, BST-2 or CD317) was identified as a restriction factor through the study of HIV accessory protein Vpu (Neil *et al*., 2008). In the presence of tetherin, Vpu-minus HIV virions are assembled normally and adopt a normal morphology. However, large numbers of the mature virions remain trapped at the surface of infected cell membrane by tetherin, and some virions are subsequently internalized, leading to retention of viral particles both at the cell surface and within the endosomes of the infected cells (Neil *et al*., 2006; Perez-Caballero *et al*., 2009). The restrictive effect occurs solely at the stage of viral particle retention rather than assembly, and these "tethered" virions are fully infectious once released. It is not yet known how tetherin "tethers" virions to the cell surface, but its unusual topology may play a key role (McNatt *et al*., 2009; Perez-Caballero *et al*., 2009). The restriction of tetherin can be counteracted by the expression of Vpu in a not fully characterized cell type-specific manner (McNatt *et al*., 2009). Tetherins can block the release of a broad spectrum of retroviruses, ranging from alpharetrovirus, betaretrovirus, deltaretrovirus, lentivirus, to the spumaretrovirus genera of retroviradae (Jouvenet *et al*., 2009).

Susceptibility of gammaretroviruses to human restriction factors

Human restriction factors have been shown to inhibit replication of gammaretroviruses. Human APOBEC3G can restrict Moloney-MLV. Human TRIM5α strongly inhibits N tropic MLV, and tetherin potently blocks the release of MLV viral particles. In addition to MLV, the block of human restriction factors to gammaretroviruses was studied in detail through investigations of their effects on a gammaretroviral xenotropic MLV-related virus, XMRV. XMRV was first isolated from patients with familial prostate cancer, and then shown to be associated with chronic fatigue syndrome (Lombardi *et al*., 2009; Urisman *et al*., 2006). Although the link between XMRV and any human disease was disproven when XMRV was shown to be a lab-derived recombinant between two endogenous murine retroviruses (Cingoz *et al*., 2012; Delviks-Frankenberry *et al*., 2012), the research on the inhibition of XMRV by human restriction factors provides us with important insights on the barriers imposed on gammaretoviruses that prevent their assuming roles as human pathogens. APOBEC3 presumably inhibits XMRV replication when single round infectivity assays were used (Groom *et al*., 2010; Paprotka *et al*., 2010). XMRV replication can be restricted by tetherin but not by human TRIM5α (Groom *et al*., 2010). Human PBMCs express APOBEC3G and 3F, as a result XMRV can infect activated PBMCs, but with little or no replication and minimal spread (Chaipan *et al*., 2011; Groom *et al*., 2010). Hypermutation of XMRV provirus from infected PBMCs is reflective of the restriction mediated by APOBEC3 (Chaipan *et al*., 2011).

KoRV, like XMRV, does not encode a Vif-like protein to escape restriction imposed by APOBEC3 proteins. KoRV infection of human PBMCs has not been reported. The different variants of KoRV we have isolated from koalas housed in US zoos are not able to infect human PBMCs following exposure to cell-free virus, even though many human cell lines including T cell lines such as SupT1 and CEM are susceptible. Human restriction factors probably play a key role in the resistance of human PBMCs to KoRV and will most likely play a major role in restricting koala retroviruses from evolving into human pathogens.

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References

Chaipan, C., K. A. Dilley, T. Paprotka, K. A. Delviks-Frankenberry, N. J. Venkatachari, W. S. Hu, and V. K. Pathak. 2011. Severe restriction of xenotropic murine leukemia virus-related virus replication and spread in cultured human peripheral blood mononuclear cells. *Journal of Virology* 85(10): 4888–4897. http://dx.doi.org/10.1128/JVI.00046-11

Cingoz, O., T. Paprotka, K. A. Delviks-Frankenberry, S. Wildt, W. S. Hu, V. K. Pathak, and J. M. Coffin. 2012. Characterization, mapping, and distribution of the two XMRV parental proviruses. *Journal of Virology* 86(1): 328–338. http://dx.doi.org/10.1128/JVI.06022-11

Delviks-Frankenberry, K., O. Cingoz, J. M. Coffin, and V. K. Pathak. 2012. Recombinant origin, contamination, and de-discovery of XMRV. *Current Opinion in Virology* 2(4): 499–507. http://dx.doi.org/10.1016/j.coviro.2012.06.009

Groom, H. C., M. W. Yap, R. P. Galao, S. J. Neil, and K. N. Bishop. 2010. Susceptibility of xenotropic murine leukemia virus-related virus (XMRV) to retroviral restriction factors. *Proceedings of the National Academy of Sciences, USA* 107(11): 5166–5171. http://dx.doi.org/10.1073/pnas.0913650107

Jouvenet, N., S. J. Neil, M. Zhadina, T. Zang, Z. Kratovac, Y. Lee, M. McNatt, T. Hatziioannou, and P. D. Bieniasz. 2009. Broad-spectrum inhibition of retroviral and filoviral particle release by tetherin. *Journal of Virology* 83(4): 1837–1844. http://dx.doi.org/10.1128/JVI.02211-08

Keckesova, Z., L. M. Ylinen, and G. J. Towers. 2004. The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proceedings of the National Academy of Sciences, USA* 101(29): 10780–10785. http://dx.doi.org/10.1073/pnas.0402474101

Kutluay, S. B., D. Perez-Caballero, and P. D. Bieniasz. 2013. Fates of retroviral core components during unrestricted and TRIM5-restricted infection. *PLoS Pathogens* 9(3): e1003214. http://dx.doi.org/10.1371/journal.ppat.1003214

Lombardi, V. C., F. W. Ruscetti, J. Das Gupta, M. A. Pfost, K. S. Hagen, D. L. Peterson, S. K. Ruscetti, R. K. Bagni, C. Petrow-Sadowski, B. Gold, M. Dean, R. H. Silverman, and J. A. Mikovits. 2009. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 326(5952): 585–589. http://dx.doi.org/10.1126/science.1179052

Malim, M. H. 2009. APOBEC proteins and intrinsic resistance to HIV-1 infection. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences* 364(1517): 675–687. http://dx.doi.org/10.1098/rstb.2008.0185

Marin, M., K. M. Rose, S. L. Kozak, and D. Kabat. 2003. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. *Nature Medicine* 9(11): 1398–1403. http://dx.doi.org/10.1038/nm946

McNatt, M. W., T. Zang, T. Hatziioannou, M. Bartlett, I. B. Fofana, W. E. Johnson, S. J. Neil, and P. D. Bieniasz. 2009. Species-specific activity of HIV-1 Vpu and positive selection of tetherin transmembrane domain variants. *PLoS Pathogens* 5(2): e1000300. http://dx.doi.org/10.1371/journal.ppat.1000300

Mehle, A., B. Strack, P. Ancuta, C. Zhang, M. McPike, and D. Gabuzda. 2004. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. *The Journal of Biological Chemistry* 279(9): 7792–7798. http://dx.doi.org/10.1074/jbc.M313093200

Neil, S. J., S. W. Eastman, N. Jouvenet, and P. D. Bieniasz. 2006. HIV-1 Vpu promotes release and prevents endocytosis of nascent retrovirus particles from the plasma membrane. *PLoS Pathogens* 2(5): e39. http://dx.doi.org/10.1371/journal.ppat.0020039

Neil, S. J., T. Zang, and P. D. Bieniasz. 2008. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451(7177): 425–430. http://dx.doi.org/10.1038/nature06553

Nisole, S., J. P. Stoye, and A. Saib. 2005. TRIM family proteins: retroviral restriction and antiviral defence. Nature reviews. *Microbiology* 3(10): 799–808. http://dx.doi.org/10.1038/nrmicro1248

Paprotka, T., N. J. Venkatachari, C. Chaipan, R. Burdick, K. A. Delviks-Frankenberry, W. S. Hu, and V. K. Pathak. 2010. Inhibition of xenotropic murine leukemia virus-related virus by APOBEC3 proteins and antiviral drugs. *Journal of Virology* 84(11): 5719–5729. http://dx.doi.org/10.1128/JVI.00134-10

Perez-Caballero, D., T. Hatziioannou, A. Yang, S. Cowan, and P. D. Bieniasz. 2005. Human tripartite motif 5alpha domains responsible for retrovirus restriction activity and specificity. *Journal of Virology* 79(14): 8969–8978. http://dx.doi.org/10.1128/JVI.79.14.8969-8978.2005

Perez-Caballero, D., T. Zang, A. Ebrahimi, M. W. McNatt, D. A. Gregory, M. C. Johnson, and P. D. Bieniasz. 2009. Tetherin inhibits HIV-1 release by directly tethering virions to cells. *Cell* 139(3): 499–511. http://dx.doi.org/10.1016/j.cell.2009.08.039

Perron, M. J., M. Stremlau, B. Song, W. Ulm, R. C. Mulligan, and J. Sodroski. 2004. TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proceedings of the National Academy of Sciences, USA* 101(32): 11827–11832. http://dx.doi.org/10.1073/pnas.0403364101

Sheehy, A. M., N. C. Gaddis, and M. H. Malim. 2003. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nature Medicine* 9(11): 1404–1407. http://dx.doi.org/10.1038/nm945

Stremlau, M., C. M. Owens, M. J. Perron, M. Kiessling, P. Autissier, and J. Sodroski. 2004. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 427(6977): 848–853. http://dx.doi.org/10.1038/nature02343

Urisman, A., R. J. Molinaro, N. Fischer, S. J. Plummer, G. Casey, E. A. Klein, K. Malathi, C. Magi-Galluzzi, R. R. Tubbs, D. Ganem, R. H. Silverman, and J. L. DeRisi. 2006. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathogens* 2(3): e25. http://dx.doi.org/10.1371/journal.ppat.0020025

Wolf, D., and S. P. Goff. 2008. Host restriction factors blocking retroviral replication. *Annual Review of Genetics* 42: 143–163. http://dx.doi.org/10.1146/annurev.genet.42.110807.091704