(Appended form No. 3)

Abstract of Doctoral Thesis

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Title: Interspecies transmission of retroviruses in ancient and modern times
(古代および現代におけるレトロウイルスの種間伝播)

Abstract of thesis:

Retroviruses are RNA viruses that infect a wide range of vertebrate hosts, include mammals. Their replication cycle is characterized by the reverse transcription of the single-stranded RNA genome into double-stranded DNA, which subsequently integrated into the host cell's chromosomal DNA. Retroviruses are mainly classified into exogenous retroviruses (exRVs) and endogenous retroviruses (ERVs). exRVs are transmitted horizontally between susceptible hosts and are associated with neoplastic and degenerative diseases. In contrast, ERVs originate from ancestral infections of germ cells and are permanently inherited within host genomes.

Among exogenous retroviruses, foamy viruses (FVs), belonging to the subfamily *Spumaretrovirinae*, are widely distributed across mammals, including cattle, horses, cats, and primates, and have zoonotic potential. FVs are complex retroviruses characterized by terminal repeats and the major genes *gag*, *pol*, and *env*, sharing structural complexity with lentiviruses. Feline foamy virus (FFV) has been reported in domestic cats worldwide, including in Asia, Australia, Germany, Turkey, and the United States. Transmission mainly occurs through salivary exchange and close physical contact, although vertical transmission via lactation has been reported in felids. FFV exists as two genotypes (F17/951-type and FUV-type), which differ in the envelope surface (SU) protein and correspond to separate neutralization serotypes. ERVs are gradually inactivated by mutations that arise during viral replication and through post-insertional mutations that occur during the replication of the host genome over time. Moreover, some ERVs undergo a process of domestication within their host organisms, thereby gradually acquiring physiological functions. ERV sequences serve as molecular fossils of past infections, enabling the study of paleovirulence and the evolutionary dynamics of host–virus interactions. With the exception of chimpanzee endogenous retrovirus 1 (CERV1)/Pan troglodytes endogenous retrovirus 1 and CERV2, all 40 ERV groups in chimpanzees are represented by lineal homologues in humans. However, although some studies have shed light on the CERV2 infection process, the detailed mechanisms of CERV1 infection remain poorly understood. This dissertation investigates how retroviruses interact and co-evolve with their hosts, focusing on ancient viral endogenization events and ongoing cross-species transmission.

In Chapter 1, the potential transmission of feline foamy virus (FFV) from domestic cats to the endangered Tsushima leopard cat (TLC), which is an endangered species found only at Tsushima Island in

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Japan were investigated. Screening of 89 TLC samples identified FFV infection in 7(7.86%) of the cats, while 14.07% of 199 domestic cats tested positive. Phylogenetic analysis showed that FFV sequences from domestic cats and TLCs clustered within the same clade, indicating shared viral strains between domestic cats and TLCs and suggesting cross-species transmission. Statistical analysis revealed no significant association between FFV infection and sex ($p = 0.28$). However, in domestic cats, FFV infection was significantly associated with co-infection with feline immunodeficiency virus (FIV) ($p = 0.002$) and feline gammaherpesvirus 1 (FcaGHV1) ($p = 0.0001$), but not with feline leukemia virus (FeLV) ($p = 0.21$). These findings highlight the importance of monitoring FFV infection in both domestic cats and TLC populations as part of conservation and disease management strategies.

In Chapter 2, the transmission and endogenization pathways of CERV1 in primate genomes were examined. Using a human cDNA library, riboflavin transporter SLC52A2 was identified as a functional cellular receptor for CERV1 entry. The ectopic expression of human and chimpanzee SLC52A2, as well as the related SLC52A1, in heterologous cells conferred susceptibility to infection by both CERV1 and porcine endogenous retrovirus (PERV). Viral interference assays demonstrated reciprocal inhibition between CERV1 and PERV, indicating that they belong to the same interference group. Furthermore, CERV1 exhibited a wide host range, infecting various human and primate cell types. Genomic analyses revealed a high prevalence of CERV1 in great apes (*Pan troglodytes*, *Pan paniscus*, and *Gorilla gorilla*) and 15 Old World monkey species, but its absent in both humans and orangutans. These findings suggest that ERVs may contribute to host resistance against viral infections through convergent evolutionary mechanisms, with important implications for paleovirology and xenotransplantation safety.

In conclusion, this study provides comprehensive insights into retrovirus–host interactions, comprising both retroviral endogenization events and ongoing cross-species transmission. The findings on CERV1 enhance our understanding of the evolutionary integration and adaptation of retroviruses within primate genomes, while the FFV results demonstrate active viral exchange between domestic and wild felids. Collectively, this work contributes to a deeper understanding of retroviral evolution, host adaptation, and the ecological and evolutionary dynamics of host–virus relationships.

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学位論文審査の結果の要旨

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審査結果の要旨：

学位論文要旨

本博士論文は、現代の生態系を脅かす「現在のレトロウイルス」と、宿主ゲノムの一部となった「古代のレトロウイルス（内在性レトロウイルス）」の双方を標的とし、それらが宿主の生存と進化に与える影響を多角的に明らかにしたものである。

第1章：ツシマヤマネコ集団および飼い猫集団におけるネコフォーミーウイルス（FFV）の疫学的調査と分子系統解析

本章では、絶滅危惧種ツシマヤマネコへの飼い猫からのFFV伝播リスクを評価した。対馬で収集したツシマヤマネコ89検体と飼い猫199検体をネステッドPCR法でスクリーニングした結果、前者で7検体（7.86%）、後者で28検体（14.07%）からFFVを検出した。 系統解析により、両集団のFFV配列が同一の系統（クレード）に集束し、極めて近縁なウイルス株を共有していることが判明した。統計解析では性別との関連はなかったが、ネコ免疫不全ウイルス（FIV）やネコガンマヘルペスウイルス1（FcaGHV1）との共感染に有意な関連が認められた。これらは飼い猫がツシマヤマネコへの感染源である可能性を示唆しており、保全医学における地域的な感染症管理の重要性を浮き彫りにしている。

第2章：チンパンジー内在性レトロウイルス1（CERV1）の細胞受容体の同定および進化史の解明

本章では、絶滅した古代ウイルスCERV1が霊長類に感染・内在化したメカニズムを調査した。ヒトcDNA発現ライブラリーを用いたスクリーニングにより、侵入受容体としてリボフラビン輸送体「SLC52A2」を同定。本来は感染耐性を持つ細胞にヒトやチンパンジー由来のSLC52A2/SLC52A1を導入すると感染が成立し、これらが侵入受容体として機能することを実証した。 さらに、ウイ

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ルス干渉実験から CERV1 はブタ内在性レトロウイルス（PERV）と同一の受容体を利用することが判明。比較ゲノム解析では、CERV1 はチンパンジーやゴリラ、旧世界ザルには存在するが、ヒトとオランウータンには欠損していた。進化解析は、約 1000 万年前に CERV1 がリボフラビン輸送体を利用して霊長類の生殖細胞ゲノムへと内在化し始めたことを示している。

まとめ

総じて本研究は、レトロウイルスが現代においては野生動物の存続を脅かす「現在進行形の脅威（第 1 章）」である一方、進化の歴史においては宿主の防御機構（生命の盾）を形作ってきた「不可欠な原動力（第 2 章）」でもあるという二面性を証明した。現代ウイルスの動態監視と古代ウイルスの機能解明から得られた知見は、野生動物保護（保全生物学）への貢献にとどまらず、ウイルスの防御機構をゲノムレベルで応用する次世代の感染症治療や医療生命科学に対し、極めて発展性の高い基盤を提供するものである。

以上により、審査委員一同は博士（獣医学）の学位論文として十分な価値を有するものと判定した。