Human endogenous retrovirus K envelope glycoprotein structures in pre- and post-fusion conformations by cryo-EM

Chen Sun[1], Jeremy Shek[1, 2], Elise M. Wilson[1,2], Fatemeh Moadab[3], Kathryn M. Hastie[1], Roshan R. Rajamanickam[1], Patrick J. Penalosa[1,2], Stephanie S. Harkins[1], Diptiben Parekh[1], Chitra Hariharan[1], Dawid S. Zyla[1], Cassandra Yu[1], Kelly C.L. Shaffer[1,2], Victoria I. Lewis[2], Ruben Diaz Avalos[1], Tomas Mustelin[3], and Erica Ollmann Saphire[1,2*]

csun@lji.org

[1]Center for Vaccine Innovation, La Jolla Institute for Immunology, La Jolla, CA 92037, USA

[2]Dept. of Medicine, University of California San Diego. La Jolla, California 92037, USA

[3]Division of Rheumatology, University of Washington, Seattle, WA 98109, USA

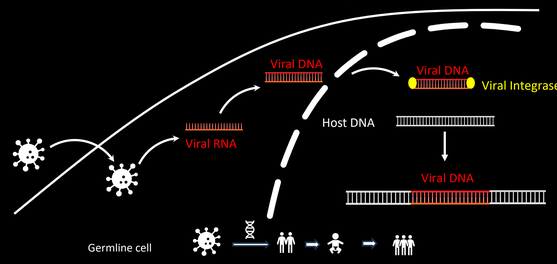
Abstract

Human endogenous retroviruses (HERVs) are remnants of ancient retroviral infections that have integrated into the germline DNA of our ancestors; HERV sequences now comprise about 8% of the human genome. Although most HERV proteins are either nonfunctional or epigenetically suppressed, the HERV-K envelope glycoprotein (Env) is expressed in numerous human diseases including cancers and autoimmune diseases and is the target of patient antibodies in these disease states. A lack of structural information on HERV Env has hindered molecular analysis and limited our understanding of its recognition by the immune system. Here, we present cryo-electron microscopy (cryo-EM) structures of the trimeric HERV-K Env protein in both its pre- and post-fusion states, each in complex with novel monoclonal antibodies. The pre-fusion Env assembles as a trimer, but with a tertiary fold and shape distinct from other retroviruses. Further, in its post-fusion six-helix bundle conformation, Env presents a unique "tether" helix within the TM subunit not seen in other retroviruses. A panel of monoclonal antibodies, elicited to facilitate Env structure determination, has been characterized for conformational specificity, SU or TM specificity, and can now be broadly used as research tools. These results and novel reagents described here establish a structural framework for mechanistic studies of HERV-K Env in diseases and evaluation as a potential target for therapeutic intervention.

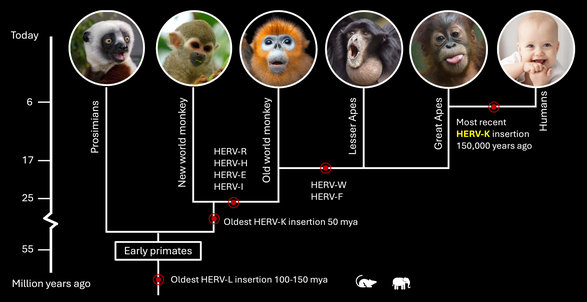
Introduction

❖ Human Endogenous Retrovirus (HERVs)

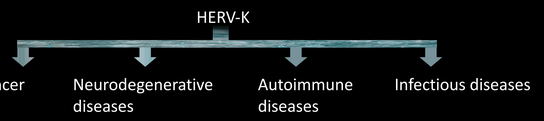
HERVs are remnants of ancient retroviral infections that integrated into the human DNA.



❖ HERV integration timeline



❖ HERV-K is associated with diseases



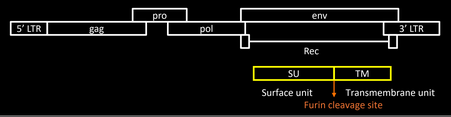
- Breast cancer
- Ovarian cancer
- Melanoma
- Germ Cell Tumors
- ...

- Amyotrophic Lateral Sclerosis (ALS)

- Multiple Sclerosis (MS)
- Systemic Lupus Erythematosus (SLE)
- Rheumatoid Arthritis (RA)
- Type 1 diabetes (T1D)

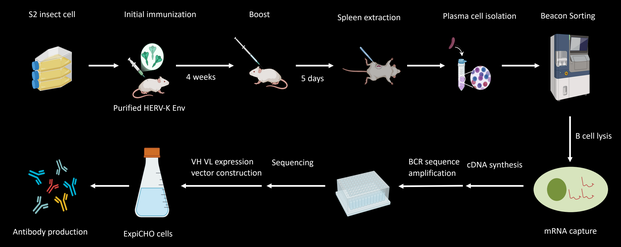
- HIV Co-Activation

❖ HERV-K (HML-2) type 2 provirus genome

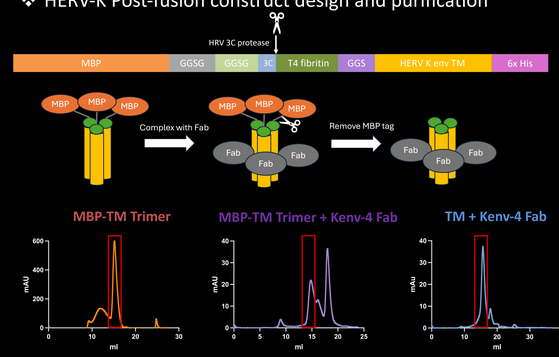


Methods

❖ HERV-K antibody discovery



❖ HERV-K Post-fusion construct design and purification



Results

❖ HERV-K Env Antibodies Stain Neutrophils in Autoimmune Patients

A

Antibody	Recognizing subunit	Epitope Type
Kenv-1	TM	Linear
Kenv-2	TM	Linear
Kenv-3	TM	Linear
Kenv-4	TM	Conformational: Post-fusion
Kenv-6	SU	Conformational: Pre-fusion
Kenv-7	TM	Linear
Kenv-8	TM	Linear
Kenv-10	SU	Linear
Kenv-12	TM	Linear
Kenv-14	SU	Linear

B

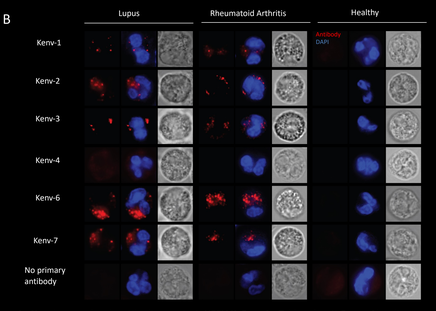


Figure 1. Novel monoclonal antibodies against HERV-K Env. A. Table of anti-Env mAbs discovered in this study. B. Immunofluorescence staining of isolated neutrophils from either SLE, RA, or healthy donors using mAbs from this study. Neutrophils from RA samples were treated with IFNα prior to fixation. Healthy neutrophils isolated with or without IFNα treatment show no staining by any mAb.

Results

❖ HERV-K Env Post-fusion structure

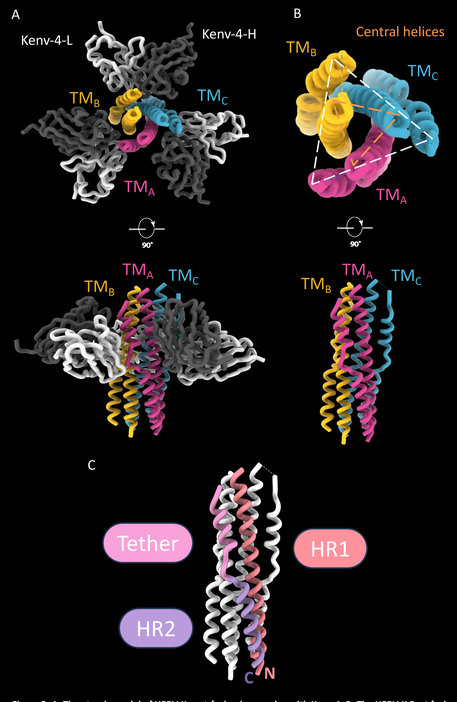


Figure 2. A. The atomic model of HERV-K post-fusion in complex with Kenv-4. B. The HERV-K Post fusion structure forms a six-helix bundle, which is the characteristic feature of all retrovirus post fusion structures. C. The center of this bundle consists of a parallel, trimeric coiled coil formed by three HR1 helices, while the HR2 and tether helices pack against the outer surface of the HR1 coiled-coil trimer in an antiparallel fashion.

❖ Sequence alignment of HERV-K TM with other retroviral fusion protein subunit sequences

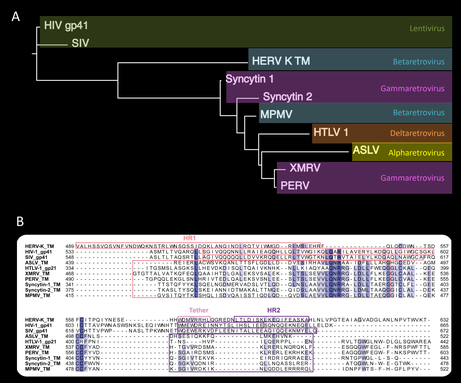


Figure 3. Comparison with other retrovirus post fusion core structures. A. Sequence alignment of HERV-K TM with other retroviral fusion protein subunit sequences. Phylogenetic tree of the compared sequences generated with NGPhylogeny.fr (https://ngphylogeny.fr/).
B. Sequence alignment of the HERV-K TM compared to HIV-1 gp41 (PDB:1I5X), SIV (PDB:1QBZ), ASLV (PDB:4JPR), HTLV-1 (PDB:1MG1), PERV (PDB:7S94), Syncytin-1 (PDB: 6RX1), Syncytin-2 (PDB: 6RX1), XMRV(PDB: 4JGS), MPMV (PDB:4JF3), performed by ClustalOmega and visualized by JalView.

Results

❖ HERV-K Post-fusion Has a Unique Tether Helix

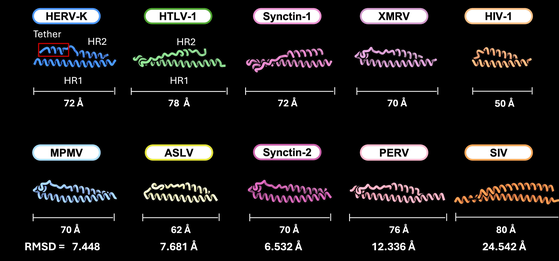


Figure 5. Comparison with other retrovirus post fusion core structures. We manually curated nine other retroviral envelope post-fusion structures from the Protein Data Bank (PDB). All structures feature a long α helix corresponding to the HR1 region. However, while other retroviruses possess only two helices (HR1 and HR2), the post-fusion HERV-K TM core exhibits an additional helix within the tether sequence, between HR1 and HR2, which we have termed the tether helix. Interestingly, the HERV-K tether is closer in sequence to other retroviruses' HR2, but it does not adopt the typical HR2 position. The residues in HERV-K HR2 are unique in sequence and do not share origin with any other retroviral proteins, although they do adopt an HR2-like packing on the outside of HR1.

❖ HERV-K Env Pre- to Post-fusion Transition

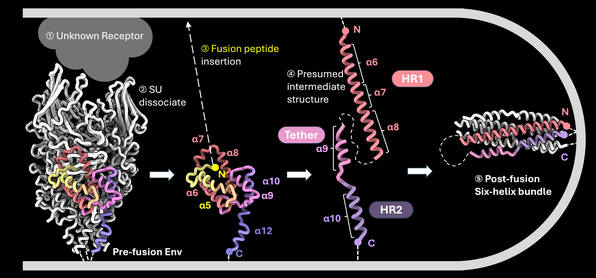


Figure 4. Conformational change of HERV-K Env from pre-fusion to post-fusion state. HERV-K Env-mediated fusion is initiated by binding to an unknown receptor which leads to the dissociation of SU from TM. The dissociation of SU from TM initiates the irreversible springing to the low energy state, where the fusion peptide inserts into the target membrane, starting the process of membrane fusion. The insertion of the fusion peptide extend α6 and α7 to form the HR1 central helix with α8, followed by a 180-degree rotation of the α9. In the end, they converge into the lowest energy state of 6-helix bundle structure and bringing the two membranes together.

Conclusions

01 We stabilized and solved the first pre-fusion structure of any HERV Env

02 The post fusion structure of HERV-K Env has a unique tether helix

03 Novel HERV-K antibodies can be used for cancer diagnosis or treatment

Antibody conjugated drugs

Bi-specific T cell engager

CAR T cell therapy

Acknowledgements

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