

FOUR LYSINE RESIDUES IN THE APOBEC3G C-TERMINAL DOMAIN ARE CRITICAL FOR HIV-1 VIF-MEDIATED UBIQUITINATION/DEGRADATION

Yasumasa Iwatani¹, Denise S.B. Chan², Lin Liu², Hiroaki Yoshii¹, Junko Shibata¹, Judith G. Levin³, Angela M. Gronenborn², and Wataru Sugiura¹

¹Clinical Research Center, National Hospital Organization, Nagoya Medical Center, Nagoya, Aichi 460-0001, Japan; ²Department of Structural Biology, University of Pittsburgh Medical School, Pittsburgh, PA 15260; ³Laboratory of Molecular Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

During coevolution with the host, HIV-1 developed the ability to hijack the cellular ubiquitin/proteasome degradation pathway to counteract the antiviral activity of APOBEC3G (A3G), a host cytidine deaminase that serves as a cellular defense protein and a potent inhibitor of HIV-1 replication. Abrogation of A3G function involves the HIV-1 Vif protein, a specific substrate receptor for ubiquitination that binds A3G. More specifically, Vif facilitates ubiquitination of A3G by forming an E3 ubiquitin ligase complex consisting of Cullin5-ElonginB/C-Rbx2-E2. Yet, despite our knowledge that these proteins interact with each other in the complex, the details of how the complex dictates A3G ubiquitination and subsequent degradation have remained unclear. Here, we report that extensive mutagenesis, guided by a structural model of full-length human A3G, has allowed us for the first time to identify four Lys residues that are required for HIV-1 Vif-dependent ubiquitination and degradation. Remarkably, substitution of Arg for Lys at these residues confers Vif resistance and restores A3G's antiviral activity even in the presence of Vif. In our model, the critical four residues cluster at the C-terminus, opposite to the known N-terminal A3G-Vif interaction region containing the ₁₂₈DPD₁₃₀ motif. Interestingly, HIV-2 and SIV_{mac} Vif proteins target the same Lys residues in A3G as HIV-1 Vif. Collectively, these findings suggest that spatial constraints imposed by the E3 ligase complex and especially the rigid scaffold Cullin5 protein may be an important determinant for Vif-dependent A3G ubiquitination/degradation.