HOST FACTORS MODULATING HIV-1 INFECTIVITY AND RESTRICTION

Dissertation
to obtain the academic degree of
Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry and Pharmacy
of Freie Universität Berlin

by

Elena Sokolskaja
from Kishinev (Moldova)

April, 2006

1st Reviewer: Dr. Jeremy Luban, Columbia University, New York

2nd Reviewer: Prof. Dr. Volker Erdmann, Freie Universität, Berlin

date of defense: 10.11.2006

Acknowledgements

Jeremy Luban's exceptional optimism and confidence in my professional ability have been crucial to my scientific achievements of the past three years. Throughout my work, his guidance, critical advice, and boundless scientific enthusiasm have made him an outstanding PhD mentor. I thank him for his constant support and for encouraging me in my development as a scientist.

I thank all members of the lab for generous sharing of reagents and knowledge. My special thanks go to Sarah Sebastian for her unintrusive yet indispensable presence, David Sayah and Lionel Berthoux for countless scientific and science-unrelated discussions, and Martha Neagu and Nam Lee for essential contribution to my extracurricular activities. I thank Jenny Drahos for providing the Cyp60-shRNA cell line, and for kindly helping with the lab move.

I thank Prof. Dr. Volker Erdmann for reviewing my thesis.

ABSTRACT

Host Factors Modulating HIV-1 Infectivity and Restriction

Retroviruses are obligate cellular parasites that depend on host cell factors for their replication. In particular, one such cellular factor, Cyclophilin A (CypA), promotes replication of Human Immunodeficiency Virus Type 1 (HIV-1) in human cells. CypA belongs to the family of peptidyl-prolyl isomerases and binds to an exposed proline-rich loop in the HIV-1 capsid protein (CA), as demonstrated by in vitro binding experiments. Although CypA has been conclusively demonstrated to facilitate HIV-1 spread in tissue culture, its role in HIV-1 replication remains unknown. Though CypA binds to mature HIV-1 CA, it is also incorporated into nascent HIV-1 virions via interactions with the CA domain of the Gag polyprotein. These findings raised the possibility that CypA might act at multiple steps of the retroviral life cycle. Disruption of the CypA incorporation into HIV-1 virions suggested that producer cell CypA was required for full virion infectivity. However, recent studies indicate that CypA within the target cell regulates HIV-1 infectivity by modulating retroviral restriction. In this study, we exploit multiple tools that disrupt the HIV-1 CA-CypA interaction to examine the relative contribution of producer cell CypA and target cell CypA to HIV-1 replication. Our results clearly demonstrate that target cell CypA, and not producer cell CypA, is important for HIV-1 CA-mediated function. Inhibition of HIV-1 infectivity resulting from virion production in the presence of CsA occurs independently of the CA-CypA interaction or even of CypA.

A recent discovery identified TRIM5α as the factor mediating retroviral

restriction in cells from mammalian species. While in non-human primates, TRIM5α potently blocks HIV-1, the human TRIM5α orthologue specifically targets N-tropic Murine Leukemia Virus (N-MLV). Paradoxically, in cells from African green monkeys and rhesus macaques, CypA is required for TRIM5α-mediated restriction of HIV-1. These observations suggest that human TRIM5α also restricts HIV-1, but only when the association of CypA and HIV-1 CA is disrupted - in other words, HIV-1 recognition by the human TRIM5α is precluded by CypA. In this study, effects of CypA and TRIM5α on HIV-1 restriction were examined directly. RNA interference (RNAi) was used to show that endogenous human TRIM5α does indeed restrict HIV-1, but the magnitude of this antiviral activity is not altered by disruption of the CA-CypA interaction or by elimination of CypA protein. Conversely, the stimulatory effect of CypA on HIV-1 infectivity was completely independent of human TRIM5α. These data suggest that TRIM5α and CypA independently regulate HIV-1 infectivity, and that in human cells CypA protects HIV-1 from an antiviral activity other then TRIM5α. In addition, cross-saturation experiments suggest that both restrictions utilize a common saturable target that is epistatic to both TRIM5α and the putative CypA-regulated restriction factor.

TABLE OF CONTENTS

CHAPTER III – CSA AFFECTS VIRUS ASSEMBLY

CHAPTER IV – RETROVIRAL RESTRICTION IN MAMMALS

CHAPTER V – TRIM5α_{hu} AND CYPA INDEPENDENTLY REGULATE HIV-1 INFECTIVITY

CHAPTER VI: CELL-TYPE SPECIFIC PHENOTYPES OF HIV-1 CA VARIANTS ARE NOT DETERMINED BY TARGET CELL TRIM5α_{hu}

LIST OF FIGURES