CHARACTERIZING THE ROLE OF CELLULAR PROTEIN ADAM10 IN HIV-1 REPLICATION

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**Background:** HIV-1 utilizes cellular proteins at several steps during replication. While some of these cellular factors are well-characterized, many cellular proteins required for HIV-1 replication still remain to be discovered. Utilizing an insertional mutagen to create libraries of mutant cells, mutation of the ADAM10 gene was found in a clone of cells surviving reovirus lytic infection. Subsequently, ADAM10 was identified to function in HIV-1 replication. **Methods:** Potential clinical relevance of ADAM10 was assessed by siRNA treatment of primary macrophages. U373-MAGI-CCR5 cells, which express β-galactosidase under control of the HIV LTR promoter, were used to help define the replication step affected by ADAM10. Virus production was assessed by ELISA 7d post-infection. New viral cDNA formation, integration, and 2-LTR circle formation were quantified by real time PCR. ADAM10 wild type and mutant plasmids were transfected into U373 cells. **Results:** In macrophages and U373 cells, transfection of ADAM10-siRNA 48h prior to HIV infection resulted in 85% reduction in HIV p24 production at 7d compared to mock treatment. Formation of full length HIV cDNA (reverse transcription) was not inhibited. β-gal production was inhibited after infection in ADAM10 siRNA-treated U373 cells indicating that the effect of ADAM10 is prior to HIV protein production. HIV cDNA did not enter the nucleus as integration and 2LTR circle formation were inhibited in ADAM10 down-regulated cells. Overexpression of ADAM10 increased infection, and mutations in the metalloprotease domain had no effect on HIV replication. **Conclusions:** The site of ADAM10 activity is after reverse transcription and before HIV integration, likely at the level of nuclear trafficking or entry. Additionally, the cytoplasmic domain of ADAM10 seems to be the functional domain associated with HIV replication. Finally, the absence of cytotoxic effects associated with down-regulation in primary macrophages suggests that ADAM10 may provide a unique target for therapeutic intervention.