

FUNCTIONAL AND STRUCTURAL STUDIES ON THE HIV-1 MATURATION INHIBITOR BEVIRIMAT

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The maturation inhibitor bevirimat (BVM) potently inhibits HIV-1 infectivity by preventing proteolytic cleavage at the CA-SP1 cleavage site in Gag. We previously isolated a panel of six single-amino-acid substitutions that confer resistance to BVM *in vitro*. The BVM-resistance mutations map to the highly conserved residues at the extreme C-terminus of CA and the N-terminus of SP1. In contrast to the SP1 N-terminus, the C-terminal portion of SP1 is not strictly conserved and contains a number of naturally occurring polymorphisms. Polymorphisms at SP1 residues 6-8 were found to correlate with variable patient responses to BVM in recent clinical studies. The HIV-1 molecular clone NL4-3 contains the clade B consensus sequence of QVT at SP1 residues 6-8. To understand the relationship between polymorphisms at residues SP1 6-8 and HIV-1 susceptibility to BVM, we introduced the following mutations into pNL4-3: Q6A, Q6H, V7A, V7M, T8A and Δ T8. Susceptibility to BVM was examined using a quantitative biochemical CA-SP1 processing assay. As expected, high levels of unprocessed CA-SP1 accumulated in wild-type (WT) virions upon BVM treatment. Similar levels of unprocessed CA-SP1 were observed in the Q6A, Q6H and T8A BVM-treated virion samples. In contrast, BVM-treated V7A virions contained levels of CA-SP1 similar to those observed in non-treated WT or a previously characterized BVM-resistant mutant (SP1-A1V). Intermediate levels of CA-SP1 processing were observed in the V7M and Δ T8 BVM-treated virions. The phenotypes observed in the biochemical analyses were confirmed in single-cycle infectivity assays and virus replication studies in Jurkat T cells. This study demonstrates that Gag polymorphisms at SP1 residues 6-8 confer varying degrees of BVM resistance. Preexisting Gag polymorphisms in SP1, together with acquired BVM-resistance mutations, thus represent a challenge for future clinical development of BVM. Further exploitation of the CA-SP1 cleavage site as an antiretroviral drug target has been hindered by a lack of high-resolution structural information of the CA-SP1 junction and the putative BVM binding pocket. To address this unresolved issue, we are using cryo-electron tomography to gain structural insights into the effect of BVM-treatment on HIV-1 virion morphogenesis and maturation.