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学位論文抄録

A small molecule compound that reduces Nef-mediated enhancement of HIV-1 infectivity
（NefのHIV-1感染性増強効果を減弱する低分子化合物）

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Abstract of the Thesis

Purpose: Nef, a multifunctional HIV-1 accessory protein, has been shown to enhance the infectivity of progeny viruses. However, the underlying molecular mechanism is still unclear. In this study, to find a clue to the molecular mechanism, we investigated whether a small molecule Nef-binding compound 2c, which we recently identified, interfered with the viral infectivity enhancement by Nef.

Methods: HIV-1 viruses were produced by transfecting proviral plasmids into 293 cells and their infectivity was assessed using TZM-bl cells as a target. The compound 2c was added to either the producer or target cells. The viral replication was assessed by using primary macrophages. The pull-down assay with glutathione S-transferase (GST) fusion proteins was also performed to further verify a direct binding of 2c to Nef.

Results: When added to viral producer 293 cells, 2c did not affect the efficiency of viral production itself, but significantly reduced the infectivity of produced viruses to TZM-bl cells. Such inhibitory activity was observed with the wild-type viruses but not with Nef-defective (ΔNef) viruses, the latter of which had significantly reduced intrinsic infectivity. Consistent with the fact that Nef was dispensable for the infectivity of vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped HIV-1, 2c did not show its inhibitory activity on the pseudotyped viruses. These results suggested that 2c reduced HIV-1 infectivity in a Nef-dependent manner. In fact, 2c also reduced the replication of the wild-type viruses in monocyte-derived macrophages but not of ΔNef viruses. Apart from 2c, a cellular tyrosine kinase, hematopoietic cell kinase (Hck) has been shown to bind Nef through its Src homology 3 (SH3) domain. The pull-down analysis with Nef-GST fusion proteins and various Hck proteins supported the model that both Hck SH3 domain and 2c directly bind Nef and their binding sites overlap.

Conclusions: Our results suggest that the direct 2c-Nef binding inhibits the interaction of Nef with unidentified cellular proteins and thereby reduces Nef-mediated infectivity enhancement.