Selection of Ankyrin Targeting HIV-1 Matrix and Identification of Its Binding Domain

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https://doi.org/10.12982/CMUJNS.2018.0024

Received: *December 6, 2017*
Revised: *March 23,2018*
Accepted: *April 19, 2018*

ABSTRACT

Ankyrin repeat protein is a novel class of non-antibody binding protein that can be applied as an alternative antiretroviral agent. Engineered ankyrin targeting the HIV-1 matrix (MA) would be a promising agent to interfere with HIV replication, since MA plays a major role in multiple processes of the viral life cycle. In this study, MA-specific ankyrin ($Ank^{GAG}G31$) was isolated from an artificial ankyrin library using a semi-automated selection process with biotinylated MA-streptavidin magnetic beads. The $Ank^{GAG}G31$-recognition site on MA was determined using both indirect and competitive ELISAs with overlapping MA tri-helical fragments and pentadecapeptides. The $Ank^{GAG}G31$ showed the highest binding signal to the MA-fragments covering helices 2-3-4 and peptides corresponding to helix 2 (residues 25-43), which were found as the target epitope. This finding was further analyzed by molecular modeling and docking. The rational models of $Ank^{GAG}G31$-MA complex indicated that the strong binding interaction was shown on helix 2 at key residues $K27^{MA}$, $K30^{M}$, and $K32^{MA}$. Taken together, the identification of the binding domain on the MA target improves our understanding of the $Ank^{GAG}G31$-MA interaction and provides the information necessary to design innovative protein targeting of the MA protein.

Keywords: Ankyrin, Binding domain, HIV-1 matrix, MA protein, Phage-displayed panning