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Screening of GPI-anchored 2P23 peptide library identified new anti-HIV peptide fusion inhibitors

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Finding a cure for HIV remains a major challenge. Gene therapy offers a promising approach, where patient cells are extracted, modified *ex vivo*, and reintroduced. One particularly effective method involves integrating constructs for membrane-anchored HIV fusion inhibitors (e.g., 2P23 and MT-C34), which block viral entry by preventing membrane fusion, thus protecting cells from infection.

Previously, our laboratory developed a construct for expressing fusion inhibitory peptides by embedding them into a short GPI-anchored CD52 molecule, thereby localizing them to the plasma membrane [1]. Cells expressing membrane-bound MT-C34 were fully protected from infection, while those with 2P23 showed weaker protection. We hypothesized that this difference could be explained by an N-glycosylation site present in MT-C34 and absent in 2P23, which may enhance membrane protein stability.

In this study, we introduced N-glycosylation motifs (NDT) at the N-, C-terminus, or both ends of 2P23. We cloned NDT-motif peptides into previously designed CD52-based constructs for membrane peptide export and delivered them to CEM/CCR5 cells via lentiviral transduction or CRISPR/Cas9-mediated knock-in into the *CXCR4* exon 2 locus. The surface levels of 2P23 and NDT-2P23 were the same, while 2P23-NDT and NDT-2P23-NDT showed a stepwise increase in membrane expression. However, only NDT-2P23-NDT achieved MT-C34-equivalent protection.

To discover improved 2P23 variants, we generated a 2P23 peptide library with randomized N-/C-terminal residues (NNK codons) and transduced it into HEK 293T/CD4/CCR5 and CEM/CCR5 cells. Library-expressing cells were FACS-sorted, challenged with replication-competent HIV, and analyzed by NGS to identify protective peptides. Twelve peptides were selected for further testing. Five peptides showed increased surface expression and higher protective levels compared to 2P23. The protective efficacy of these peptides will be further evaluated in infection tests with replication-competent HIV.

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References

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