Секция «Вирусология»

Engineering of genetically-modified cells secreting the HIV-1 fusion inhibitory peptide MT-C34 into the extracellular space

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HIV-1 is a human lentivirus that infects CD4-positive immune cells and when left untreated, it manifests in the fatal disease known as AIDS. One way to control the infection is to increase the population of HIV-1 -resistant CD4 lymphocytes. It was reported that peptides from the heptad repeat (HR) domain of HIV-1 gp41 being expressed on the cell surface are potent inhibitors of HIV-1 fusion with the host cell membrane [2].

Previously in our lab, HIV-1-resistant T cells were generated using CRISPR/Cas9-based fusion inhibitory peptide knock-in (KI) technology [3]. A series of HIV-1 fusion inhibitory peptides were embedded in CD52, the shortest glycosylphosphatidylinositol (GPI)-anchored protein. One of the tested peptides, membrane-bound fusion inhibitory peptide MT-C34 exhibited significant activity against both cell-free and cell-to-cell HIV-1 infection.

The aim of this study was to generate genetically modified cells for the expression of a secreted variant of MT-C34 to prevent HIV-1 infection not only in the modified cells but also in the non-modified neighboring cells. To provide the short peptide secretion, two peptides MT-C34 were linked via a cleavage site recognized by the cellular protein convertase furin [1]. Lentiviral vectors for the expression of such concatemers were created and used for the generation of cell lines HEK293T/MT-C34-1 and HEK293/MT-C34-2. The first cell line released the fusion inhibitory peptide MT-C34 into the extracellular space, the second one expressed both the secreted and membrane-bound variant of this peptide.

Infectivity assay of cells and supernatants of the two cell cultures have demonstrated that one of the secreted peptides MT-C34 which is produced after concatemer cleavage by furin as well as the membrane-anchored peptide MT-C34 provide a strong anti-HIV-1 protective effect and inhibit HIV-1 pseudovirus entry into the permissive HEK293T/CD4/R5 cells. Thus, the expression constructions and the engineered cell lines for secreting the fusion inhibitory peptide MT-C34 can be used for developing the peptide-based therapy of HIV-1 infection.

This research was funded by the Ministry of Science and Higher Education of the Russian Federation, grant number 075-15-2019-1661.

References

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