Poster

Small glycomimetic serves as antagonist to viral glycoprotein HCMV UL141 to inhibit the TRAIL death receptor binding

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The human cytomegalovirus (HCMV) persists indefinitely despite vigorous humoral and cellular immune reactions. This virus poses a substantial threat to the well-being and survival of immunocompromised persons, including transplant recipients and individuals with HIV, as well as those affected by congenital infection. Therefore, a vaccine against HCMV is considered to be the highest priority, particularly for the prevention of congenital disease, but none has been licensed yet. NK cells are crucial for virus control resulting in HCMV having an impressive arsenal of encoded immune evasins that act to suppress NK cell activation through the manipulation of ligands for activating and inhibitory receptors. HCMV UL141 restricts the surface expression of CD155 and CD122, which activate NK via DNAM-1 and CD94. In addition, UL141 also binds and inhibits expression of the TRAIL death receptors to block NK- mediated killing by this TNF-family cytokine, highlighting the poly-functionality that a single HCMV immunomodulatory protein can have. Moreover, HCMV UL141 was reported to affects viral DNA replication while interacting with CELF5. Having these several unique properties UL141 keeps HCMV in charge, thus is considered a multi-faced viral protein that has proven to be a desirable target for development of novel therapeutics designed to inhibit viral entry and spread.

The aim is to develop the short peptide or synthetic compound (UL141 antagonist) based on our recent crystal structure and computational design that would specifically bind viral UL141 to block receptor binding thus prevent the viral action. This is relevant, as the UL141 is also the most abundant HCMV protein on plasma membrane and it is also a component of the virion. Based on our computational screening of iminosugars the 'hit' structure was selected. We test a small library of synthetized compounds (potential UL141 antagonists) that would block the receptor binding in vitro, on the cell or virion surface. Series of compounds that have been tested are of glycomimetic structures consisting of various saccharide units linked with non-saccharide.

In particular, non-ionic glycolipids, 'click'-conjugates or iminosugars. The ELISA-like TMB assay has been used in combination with dynabeads coating to test whether the compound could block the TRAIL-R2 binding. Five most promising compounds have proven the ability to block UL141/TRAIL-R2 complex formation. SPR kinetics analysis was then used to determine the binding constants (K_D). The affinities to UL141 were determined in low micromolar scale. Three potential binding sites were revealed by molecular docking on UL141 surface. Next, mutational analysis on UL141 protein and subsequent SPR binding analysis showed two mutants lost the ability to block the TRAIL-R2 binding thus revealed the binding site of novel antagonist. The successful compounds will be further optimized by using *in silico* methods to target epitope on viral glycoprotein UL141 derived from our structural analysis and will be tested *in vivo* for HCMV inhibition.

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