Proposal: Designing and testing the efficacy of a peptide mimetic of phosphatidylinositol-3-kinase's SH3 domain on inhibiting influenza virus' replication and infection

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Research Objectives:

1. To design a stable and efficient peptide mimetic of the SH3 domain from phosphatidylinositol-3-kinase and to attach it to a human carrier protein
2. To measure potential cytotoxic effects of the novel therapeutic peptide mimetic
3. To test the efficacy of peptide mimetic in inhibiting the replication and infection of *Influenza A H1N1* virus

Background:

Influenza is a respiratory virus, which affects approximately 5-10% of adults and 20-30% of children worldwide (1, 2). Influenza infection is mainly characterized by a sudden onset of symptoms such as fever, headache, cough, sore throat, myalgia, nasal congestion, weakness, and loss of appetite (1, 3). Of the three main types of influenza virus (A, B, and C), influenza A is mainly responsible for causing the seasonal flu outbreaks and occasionally, results in pandemics (4) This can result in hospitalization and death in high-risk groups such as the very young, the elderly, and or chronically ill (2, 5). The World Health Organization estimates there are approximately three to five million annual cases of severe influenza worldwide, which end up claiming 250,000 to 500,000 lives (2, 4). In 2003, in the United States alone, it was estimated that the total economic costs caused by the influenza epidemic due to deaths, hospitalization fees, and loss of productivity amounted to $87.1 billion (5). Influenza is also capable of causing pandemics. In the 20th century, there were three documented cases: the 1918 Spanish flu, the 1957 Asian flu, and the 1968 Hong Kong Flu, which collectively resulted in more than 60 million deaths (6). Currently, vaccination is the best method for controlling both the seasonal and pandemic influenza virus outbreaks (4). However, it takes a significant period of time to develop these vaccines and as such, antiviral drugs are required in the meantime for treating individuals with little or no prior immunity (4). The two main classes of antiviral drugs used for influenza are: adamantanes (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir and zanamivir) (4). However, with the emergence of oseltamivir resistant and adamantane resistant influenza strains, there is a need for the development of novel anti-influenza drugs (4).

The influenza virus is classified within the *Orthomyxoviridae* family, and consists of a negative-sense, single-stranded RNA genome (4, 5). Its genome is comprised of eight segments, which encode for 11 to 12 viral proteins (5). Of these viral proteins, one of them is called nonstructural protein 1 (NS1), which modulates virus infection and antagonizes the host's innate immune system (5, 7). NS1 interferes with the phosphatidylinositol 3-kinase (PI3K)/Akt

pathway, which is linked to cell survival and proliferation (7). It has been shown that NS1 interacts directly with the p85β subunit of PI3K through binding directly to the Src homology 3 (SH3) domain of p85β (7). This leads to the activation of the PI3K pathway, which promotes virus entry, viral RNA expression, and preventing premature apoptosis of the host (7). It has been previously shown in scientific literature that heterologous overexpression of the SH3 domain can inhibit influenza virus replication by interfering with the interaction between NS1 and the host cell's native p85β subunit of PI3K (7). In this case, the SH3 domain acts as a dominant negative mutant, also referred to as a peptide mimetic. A dominant negative mutant encodes for a gene product that can bind to the same substrate as the wild-type enzyme, but it lacks the ability to catalyze reactions (8). Thus, the heterologous expressed SH3 domain will compete with native SH3 domain of p85β of PI3K for NS1 binding. However, the heterologous SH3 domain will not contribute to the downstream signalling of PI3K and hence, will not assist influenza virus infection and replication.

The purpose of my project is to expand on the work done by previous studies. Instead of transfecting a vector containing the SH3 domain (7), I am planning to design a stable and efficient peptide mimetic of the SH3 domain from phosphatidylinositol-3-kinase fused to a human carrier protein. I plan to fuse the SH3 domain to the monomeric IgG1 Fc, which will protect the peptide mimetic from degradation and increase its serum half-life (9). Furthermore, a cell penetrating peptide derived from the HIV-1 Tat Nuclear Localization Signal (NLS) will be included in this peptide mimetic. This will allow the peptide mimetic to enter into the cell's nucleus where it can interact with influenza's NS1 and help inhibit influenza replication and infection. The effectiveness of the proposed treatment will be assessed through a challenge test with 2009 Pandemic influenza A H1N1 virus. The outcome measures for drug efficacy will be determined by levels of viral load in Madin-Darby canine kidney cells after administration of the peptide mimetic. The outcome measures for the drug's cytotoxicity will be assessed by using an ATP-based luminescence assay and its effect on cell replication.

If these *in vitro* experiments produce promising results, the next step would be to proceed to doing *in vivo* experiments on a mouse model of influenza infection. The therapeutic peptide can be administered through a nasal spray and hen the mice will be challenged with influenza virus. The effectiveness of this treatment can then be assessed by comparing the viral loads between peptide mimetic treated mice and control mice. Nevertheless, this therapeutic peptide will introduce a new antiviral drug to the market and help combat the effects of seasonal and/or pandemic influenza virus.

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