A novel screening using the psudo-viral genome for the virus inhibitors

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Influenza virus type A (IAV) and human parainfluenza virus (hPIV) are important respiratory tract pathogens. IAV spreads around the world in seasonal epidemics, resulting in the deaths of between 250,000 and 500,000 people every year. Antiviral drugs such as the neuraminidase inhibitor, for example oseltamivir, can be used to treat IAV, however there is the key problem of emerging resistant virus against there drugs. hPIVs are the second most common cause of lower respiratory tract infection in younger children. In immunosuppressed people, such as transplant patients, or in young children, hPIV infections can cause severe pneumonia, which can be fatal. There is no anti-virus drug against hPIV1, and the drugs will be more important because of low birthrate and longevity in the world.

We are researching to establish the novel screening system using the green fluorescence protein (GFP) for the IAV or hPIV inhibitors. Existing screenings have a disadvantage in the culture time or necessary of specific anti-virus antibodies. Thus, these assays are not unsuitable for the screening of a lot of compounds and in short order. We are establishing competitive screening with GFP gene incorporation into virus or host cell genome, which can easily calculate the virus infection and growth as the fluorescence of GFP. With this screening, the anti-viral activity of more drug candidate compounds will be estimated more rapidly.

To establish the screening, we constructed the two types of recombinant expression plasmid vectors, polI-IAV-NP-GFP, which contains anti-sense GFP gene pinched between the 5'- and 3'- noncoding regions of the IAV nucleoprotein gene sequence, and polI-hPIV3-le-GFP-tr, which contains anti-sense GFP gene pinched between the leader sequence and the trailer sequence of the hPIV3 genome. Non-replicative GFP-expression recombinant IAV will be able to be constructed with polI-IAV-NP-GFP and the other 7 viral genes, and psudo-viral genome-induced cell line will be able to be established with polI-hPIV3-le-GFP-tr. These plasmids may be useful for the anti-viral drug screening.