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Identification of New Druggable Antiviral Targets by Chemical Genetics

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Viral infection is a continuing threat to mankind and drug resistance to available antivirals has appeared in an alarming rate. There is an urgent need to identify new druggable targets and find new antiviral drugs in light of rapid emerging and re-emerging viral pathogens. Our first success in applying forward chemical genetics in the identification of biologically active small molecule inhibitors of SARS-CoV in 2004 (Kao et al., Chemistry & Biology, 11:1293) has prompted us to tackle influenza A virus pathogenesis using this novel approach. Applying the concept of chemical genetics, after screening a validated high quality chemical library (50,240 structurally diverse small molecules) with an automated robotic platform for high-throughput screening (HTS), we have identified more than 1000 small-molecule inhibitors that will inhibit the infectivity of the viruses in Madin-Darby canine kidney (MDCK) cell-based HTS assay. Subsequent secondary screening and hit validation processes identified 39 potent antiviral compounds interfering with influenza A infection. We further identified compounds that perturbed intracellular trafficking of the viral nucleoprotein (NP) and characterized a compound (nucleozin) that apparently stopped the nuclear localization of the NP (Kao et al., Nature Biotechnology 28:600). The binding site of compound nucleozin was mapped to residue Y289 of NP. Balb-c mice treated by nucleozin were significantly protected after infection by a hypervirulent strain of influenza A H5N1/Vietnam/1194/04, illustrating the *in vivo* efficacy of nucleozin in inhibiting H5N1 infection. Further investigation using immunofluorescence techniques and gel-shift assays illustrated that nucleozin induced a time-dependent and RNA enhanced specific aggregation of NP. Our results demonstrated that chemical genetics is an attractive approach for the identification of druggable targets, new antiviral drugs, and novel antiviral mechanisms.

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Activities of Viral M2 Channel, Neuraminidase, and RNA Polymerase Inhibitors on Oseltamivir-Resistant H275Y Influenza A (H1N1) Virus Infections in Mice

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A novel influenza infection model was developed by serially passaging influenza A/Mississippi/3/2001 (H1N1) H275Y virus seven times in mice to increase its virulence. The viral neuraminidase was sensitive to inhibition by zanamivir, but had reduced susceptibility to peramivir, and was resistant to oseltamivir carboxylate, with IC_{50} values of 1, 39, and 100 nM, respectively. Several compounds were evaluated against lethal A/Mississippi H275Y virus infections in mice. Treatments with amantadine or rimantadine (viral M2 channel blockers), oseltamivir or zanamivir (viral neuraminidase inhibitors), or ribavirin (viral RNA polymerase inhibitor) were initiated 2 h before or 24 h after intranasal virus challenge, continuing twice daily for 5 days. Oral oseltamivir treatment at 1–30 mg/(kg day) was ineffective, whereas treatment with 100 and 300 mg/(kg day) gave 30 and 60% protection from death, respectively, starting at −2 h. All doses of oseltamivir were inactive starting at +24 h. Intraperitoneal treatments with zanamivir at 100 and 300 mg/(kg day) gave 60 and 90% protection, respectively, starting at −2 h. Zanamivir failed to protect mice when treatments were initiated at +24 h. The results with zanamivir were unexpected, based upon its potency in cell culture. Oral treatments with the other inhibitors were initiated at −2 h. Amantadine was effective at 10, 30, and 100 mg/(kg day), rimantadine was protective at 10 and 30 mg/(kg day) (100 mg/(kg day) was not tested), and ribavirin was active at 30 and 75 mg/(kg day), with survival ranging from 60 to 100%. Treatment with these agents was also effective when begun at +24 h. Here, amantadine activity was present at 30 and 100 mg/(kg day), rimantadine showed efficacy at 10 and 30 mg/(kg day), and ribavirin was protective at 75 mg/(kg day), with 60–100% survival for each group. These results are important in establishing this model for evaluation of drug combinations against this oseltamivir-resistant virus.

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CMX001 (Hexadecyloxypropyl Cidofovir) Antiviral Activity against Adenovirus in Patients Correlates with Drug Levels and Viral Sensitivity

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Background: Adenoviruses (AdV) are double-stranded DNA viruses; there are at least 52 distinct types. CMX001 is a lipid derivative of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC, CDV) with potent antiviral activity against AdV in vitro. CMX001 has been used to treat patients with severe AdV infections under Emergency Investigational New Drug Applications. Notably, most patients had previous exposure to cidofovir (CDV). Here we investigate the effects of drug levels and AdV drug sensitivity on the activity of CMX001.

Methods: We analyzed 22 patients with AdV viremia who had received at least 2 doses of CMX001, had measurable plasma viremia at baseline, and had viral load data available at ≥2 weeks after the first dose of CMX001. Phenotypic resistance to CDV and CMX001 was determined using a cytoprotection assay in HeLa cells. Noncompartmental pharmacokinetic (PK) analysis was conducted using plasma concentration data obtained after the first dose of CMX001.

Results: There was a median $-2.5\ \log_{10}$ change in viremia from baseline by the last timepoint (range -6.0 to $+0.3\ \log_{10}$). Patients who had either sensitive virus or no proven resistance had a median decrease of almost 1000-fold ($-2.9\ \log_{10}$) from baseline while those with CDV resistance (all >10 fold relative to wild-type [$n = 6$]), attributed to prior suboptimal CDV therapy, had a median $-0.55\ \log_{10}$ decrease from baseline. Patients with both virologic and PK data (13 of 22) were evaluated by comparing those with ($n = 10$) or without ($n = 3$) $a > 1\ \log_{10}$ reduction in viremia at week 2. The median exposure (AUC0-inf) was 1.7-fold higher (3485 vs 2047 h ng/mL) and Cmax was more than 2-fold higher (387 ng/mL vs 153 ng/mL) in patients with $a > 1\ \log_{10}$ reduction in viremia at week 2.