resulted in tumor growth suppression. EBNA1 plays a key role in EBV's gene transcription and since EBV is maintained in the nucleus of the infected cell, EBNA1 has access to host genomic DNA, as well. Therefore, we examined whether it may bind to cellular sequences. Here we report testing this hypothesis in EBV-free cell lines which are engineered to express EBNA1. Initially, microarray analysis demonstrated changes in gene expression as a reflection of EBNA1's expression. Subsequently, an anti-EBNA1 antibody was used to produce chromatin immunoprecipitation DNA from these cell lines. These DNA samples were hybridized to human promoter arrays to reveal that host genomic sites had been complexed with EBNA1. These genomic binding sites consisted of coding and non-coding sequences from both DNA strands. We have managed to demonstrate the presence of the promoters for some of the genes depicted by the microarray expression analysis, and to confirm it by QR-PCR. Subsequently, we have identified an EBNA1 consensus sequence within the engaged cellular promoters. This sequence shows no similarity to the viral DNA binding sequence of EBNA1 or to any transcription factor. This dual transcription capacity improves the virus' ability to coexist within the infected cell by monitoring viral gene expression while actively and directly manipulating host gene expression. Our studies corroborate evidence of the crucial role EBNA1 plays in EBV etiology and neoplasm and suggest that a genomic approach is needed to monitor drug design directed to target EBNA1 as it is involved in various cellular gene expression changes. Currently, the lack of specific anti-EBV therapies generates adverse side effects for patients undergoing chemotherapy.

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Oral Session 5: Respiratory Viruses, Emerging Viruses and Biodefense

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Plenary: Filoviral Minigenome systems and iVLPs as tools for antiviral research

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Identification and Mechanistic Studies on a Novel Class of Influenza Virus Fusion Inhibitors

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We here report on a new class of inhibitors of influenza virus hemagglutinin (HA)-mediated fusion, with a similar backbone structure as some reported fusion inhibitors, consisting of an aromatic cyclic system linked to a non-aromatic cyclic system via an amide bridge [Luo et al., Virology, 226:66–76 (1996)]. In Madin–Darby canine kidney (MDCK) cells infected with influenza virus A/H3N2 (X-31), the 50% effective concentration of the lead compound [4M] was 3.4 μM, as determined by microscopic examination of the viral cytopathic effect and MTS cell viability assay. The concentration producing 50% inhibition of cell proliferation was 89 μM. Similar activity was seen for other A/H3N2 strains, whereas no activity was noted for influenza A/H1N1 and B viruses. At 20 μM [4M], virus yield was reduced by 3 logs. In time-of-addition studies, [4M] lost activity when added 1 h or later post-infection, showing that [4M] inhibits an early step in virus replication. Definite proof for HA-mediated fusion as the antiviral target was provided by the inhibitory effect of [4M] on virus-induced red blood cell hemolysis at low pH. [4M]-resistant mutants, selected after three passages in MDCK cells in the presence of 20–150 μM [4M], were plaque-purified and sequenced. The two main amino acid substitutions associated with [4M] resistance were R220S and E57K, located in the HA1 and HA2 domain, respectively. This suggests that [4M] may bind to the same pocket of the influenza virus HA as the structurally unrelated H3-specific fusion inhibitor tert-butyl hydroquinone [Russell et al., PNAS, 105:17736–41 (2008)]. The mutants showed uncompromised fitness, with similar efficiency for binding and replication as wild-type virus. However, in the hemolysis assay, the mutants displayed an increased fusion pH. Molecular modelling of [4M] within the HA structure will help to design new [4M] derivatives with improved activity. Also, the activity against other virus subtypes (e.g. A/H5N1 and A/H7N7) is under investigation.

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Novel Broad-Spectrum Biopharmaceuticals: From HIV-1 to Pandemic Influenza A Virus

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Recently, my laboratory has reported the identification of the most potent endogenous furin-directed inhibitor, Spn4A [K_i: 13 pM (Richer M. et al. PNAS 2004)]. Because of our previous success with the bio-engineered serpin α_1-PDX [K_i; 0.6 nM; (Jean F. et al. PNAS 1998)] as a protein-based therapeutic (Jean F. et al. PNAS 2000), we hypothesized that strategic manipulation of the furin-like cellular subtilase levels by Spn4A and Spn4A-engineered variants might provide a means of effectively inhibiting the subtilase-dependent proteolytic cleavage of viral envelope precursor glycoproteins in the host secretory pathway, a critical cellular event required for production of infectious progeny (e.g., HIV-1, highly pathogenic H5N1 influenza A virus, West Nile virus, Dengue virus).

In this study, we report our recent original work in the exciting field of protein-based inhibitors as broad-spectrum biopharmaceuticals. First, we describe the anti-proteolytic activities and anti-HIV properties of our novel recombinant adenovirus (Ad−) expressing Spn4A variants (Ad-Spn4A) in the host cell secretory pathway. We demonstrated that expression of Ad-Spn4A in MAGI-CCR5 cells completely inhibited the subtilase-dependent processing of the HIV-1 envelope precursor gp160 and resulted in a complete reduction of productive HIV-1 infection as determined by HIV-1 Tat-driven β-galactosidase activity and syncytia formation assays. Second, we demonstrated that our novel Ad-Spn4A variants also completely block the subtilase-mediated cleavage of the hemagglutinin H5 encoded by pandemic influenza A viruses (HK/97) and resulted in a complete block of syncytia formation in human A549 epithelial cells. The detailed cellular mechanism of action of our novel serpin-based antiviral strategy and the impact of our findings for developing a novel generation of broad-spectrum protein-based