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Novel pan-antiviral strategy and delivery using gold nanoparticles for inhibiting growth of all influenza viruses including the drug-resistant seasonal human, avian H5N1 viruses, and 1918 pandemic influenza virus

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Abstract – Annual influenza epidemics caused by influenza A and B viruses result in approximately five million cases of severe illness every year. The present investigation proposes a new therapeutic method and delivery system through electrostatic coupling of gold nanoparticles to 5'PPP-containing ssRNA (Fig. 1) that target the host antiviral response in human bronchial epithelial cells (A549). Plasmon enhanced dark field imaging was used to visualize the uptake of these nanoplexes *in vitro* (Fig. 2). Activation of RIG-I pathway resulted in up-regulation of type 1 interferon and a decrease in influenza viral replication following uptake of our gold nanoplexes into A549 cells.

Annual influenza epidemics caused by influenza A and B viruses result in three to five million cases of severe illness with about 250,000 to 500,000 deaths globally every year. With growing resistance to current antivirals such as amantadine and rimantadine, there is an increasing need to investigate novel antiviral and delivery strategies.

Gold nanoparticles (GNPs) and nanorods (GNRs) have gained increasing interest as site-specific carriers of various diagnostic and therapeutic agents, primarily owing to their biocompatibility[1]. Their surfaces can be easily modified to incorporate cationic charges, which facilitate their stable electrostatic complexation with anionic genetic materials such as siRNA and ssRNA, for the purpose of targeted gene delivery and expression. Moreover, by exploiting the phenomenon of surface plasmon resonance (SPR) associated with GNPs/GNRs, their complexation with genetic materials and subsequently their delivery and distribution within target tissues can be monitored.

We propose a new therapeutic method and delivery system through electrostatic coupling of gold nanoparticles to 5'PPP-containing ssRNA. The presence of viral RNA in the cytosol is recognized by RIG-I, whereby upon interaction with either double stranded RNA or 5'PPP-ssRNA results in induction of anti-viral type 1 interferon response. We confirmed the formation of the nanoplexes from the restricted electrophoretic mobility of the nanoplexes using gel electrophoresis (Fig.1). The uptake of the nanoplexes into A549 human bronchial epithelial cells *in vitro* was determined using plasmonic enhanced dark-field imaging of gold nanorods, but also from confocal microscopy (Fig.2a) and fluorimetric analysis of cell lysates (Fig.2b). Moreover, we observed that the delivery of the nanoplex involving 5'PPP-ssRNA in A549 cells resulted in increased gene expression of RIG-I and IFN-β, and successful transfection of these nanoplexes into A549 cells resulted in reduction of WSN and 2009 H1N1 "swine influenza" viral replication. Hence, the aforementioned technology could provide a therapeutic and prophylactic alternative to seasonal and pandemic influenza.

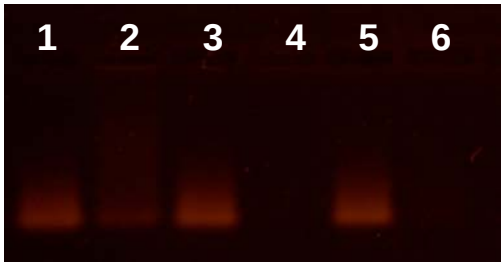

Fig.1

Figure 1: Binding efficiency of 5'PPP with GNR studied by agarose gel electrophoresis. Results show retarded mobility of the nanoplex (5'PPP/GNR) upon complexation with increasing amounts of GNRs Gel A: ethidium bromide staining UV lane 1, 3, 5: 5'PPP (30ng), lane2: GNR-5'PPP (250 ng GNR/30ng 5'PPP), lane4: GNR-5'PPP (500 ng GNR/30ng 5' PPP), lane6: GNR-siRNA (750 ng GNR/30ng 5'PPP)

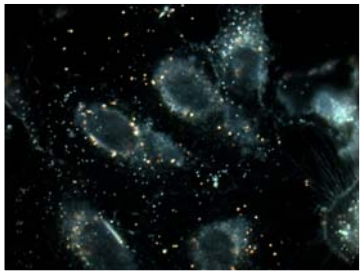

Fig.2a

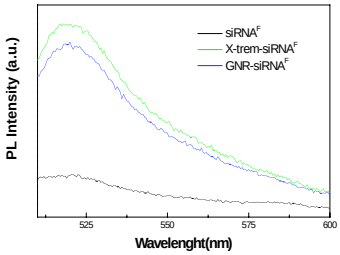

Fig.2b

Figure 2: Qualitative uptake of nanoplexes in A459 cells was monitored by dark field and confocal microscopy. Cells nucleus was stained with hoecst. Nanoplexes are can be easily observed from the strong orange-red scattering of GNR and cells nucleus stained with Hoecst. (2a) Quantitative uptake of nanoplexes in A459 cells was investigated using spectrophotometric measurements. Data shows transfection with the GNR-siRNAF nanoplexes result in the highest cellular uptake. (2b)

References:

1. Ghosh, P., et al., *Gold nanoparticles in delivery applications.* Adv Drug Deliv Rev, 2008. **60**(11): p. 1307-15.