Influenza A Virus NS1 Protein Targets Double Stranded RNA-binding Protein PACT to Suppress Innate Antiviral Response

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Background/Objective
Influenza A virus (IAV) is a common human pathogen causing devastating pandemics and seasonal epidemics. Innate antiviral immune response exemplified by the production of type I interferons (IFNs) is induced when IAV is recognized by Toll-like receptors and RIG-I-like receptors. To ensure its successful infection and replication, IAV encodes IFN-antagonizing non-structural protein NS1, a multi-functional double-stranded RNA binding protein that interacts with both RIG-I and PKR to circumvent host antiviral defense. RIG-I is a cytosolic sensor of IAV. We have previously shown that RIG-I requires PACT for full activity. PACT is a cellular dsRNA-binding protein originally identified to be an activator of PKR. Here we study how IAV NS1 might affect the function of PACT in innate antiviral response.

Method
We performed overexpression and knockdown experiments in cultured cells to study the counteracting functions and properties of PACT and NS1. We also prepared wildtype and Delta-NS1 IAV by reverse genetics. We characterized the role of PACT in NS1-mediated suppression of RIG-I-induced IFN response by reporter assay. RNA-nucleoprotein complex (RNP) reconstitution assay was performed to study the effect of PACT on RNP activity.

Result
NS1 physically interacts with PACT to perturb RIG-I-dependent IFN production. PACT-mediated potentiation of RIG-I activation on IFN-ß reporter activity was significantly inhibited by NS1. PACT-knockdown in cells promoted IAV replication. PACT acts as an inhibitor of IAV polymerase transcriptional activity. Overexpression of PACT could significantly dampen the RNP activity. The observed PACT-mediated suppression of RNP activity could be partially rescued by overexpression of NS1 protein. Furthermore, PACT-mediated suppression of RNP activity is IFN independent.

Conclusion
PACT is a novel target of IAV IFN-antagonizing protein NS1. PACT and NS1 have counteracting roles during IAV infection. PACT has an IFN-independent effector function on counteracting IAV replication. Our findings provide new insights into the inhibitory mechanism of PACT on IAV replication.