Mass-spectrometry based profiling of PKR-interaction partners identifies KSRP as a novel protein regulator of PKR

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Introduction
The RNA-dependent protein kinase (PKR) is an interferon inducible, double-strand RNA-activated protein kinase that plays a significant role in innate antiviral immunity. Due to its key role in antiviral immunity, many viruses have evolved mechanisms to avoid PKR-activated effects. Virus and others have previously described the influenza virus non-structural protein 1 (NS1) as an antagonist of PKR. We are interested in further elucidating the role of cellular and viral factors in regulating PKR activation in the context of influenza virus infection.

We used a SILAC approach followed by LC-MS/MS analysis to identify precipitable interaction partners of PKR upon influenza A virus (IAV) wildtype or ΔNS1 infection and validated selected binding partners in independent biochemical assays.

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Methods

Bioinformatic evaluation and validation

KSRP is a protein regulator of PKR

Conclusion
Taken together, this study demonstrates the aptitude of quantitative mass spectrometry for elucidation of cellular antiviral response pathways. Precipitation of PKR and binding partners revealed KSRP as a novel protein regulator of PKR. The mechanism of KSRP mediated PKR activation seems to be complex and needs to be analyzed in greater detail in ongoing studies.

Fig. 1 The RNA-dependent Protein Kinase (PKR).
PKR is an antiviral effector protein. Upon recognition of viral RNA it leads to inhibition of translation, apoptosis and production of type I IFN. The NS1 protein of influenza A and B viruses can block PKR activation.

Fig. 2 Experimental setup.

Fig. 3 Bioinformatic analysis of GFP-PKR bound proteins and exemplary validation by CoIl.

Fig. 4 Overexpression of identified PKR interaction partners induces phosphorylation of endogenous PKR.
A. Overexpression of Protein 2, 5, 7 or KSRP induces phosphorylation of endogenous PKR in mock and WT IAV infected HEK 293T cells in comparison to negative control (NC), plasmid vector pcDNA transfected cells.

Cells transfected with the known protein activator of PKR PACT serve as positive control. B. Quantification of phospho-PKR levels after mock infection and normalization on actin levels confirm the influence of Protein 2, 5, 7 and KSRP on PKR activity. Values represent the x-fold change of band intensities of immunoblots compared to NC with x-fold change of positive control PACT set to 1.

Fig. 5 KSRP interacts constitutively with PKR and is able to enhance PKR phosphorylation, which can negatively affect viral replication.
A. PKR and KSRP bind constitutively in non-infected and WT or mutant IAV infected human alveolar epithelial cells as seen by coprecipitation analysis of endogenous proteins. B. Overexpression of KSRP in HEK 293T cells induces phosphorylation of endogenous PKR in a dose-dependent manner. C + D. Viral replication of IAV mutants ΔNS1 and R46A, that are not able to inhibit PKR activation, is significantly enhanced after knockdown of KSRP in human alveolar epithelial cells treated with T6A/IRK+ KSRP inhibitor B795, to exclude effects caused by type I interferon expression. KSRP knockdown and effect of inhibitor were confirmed at 72 hpi by SDS-PAGE and immunoblotting using the indicated antibodies.

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