Hepatitis C virus NS3-4A inhibits the peroxisomal MAVS-dependent antiviral signalling response

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Abstract

Hepatitis C virus (HCV) is the cause of one of the most prevalent viral infections worldwide. Upon infection, the HCV genome activates the RIG-I-MAVS signalling pathway leading to the production of direct antiviral effectors which prevent important steps in viral propagation. MAVS localizes at peroxisomes and mitochondria and coordinates the activation of an effective antiviral response: peroxisomal MAVS is responsible for a rapid but short-termed antiviral response, while the mitochondrial MAVS is associated with the activation of a stable response with delayed kinetics[1,2]. The HCV NS3-4A protease was shown to specifically cleave the mitochondrial MAVS, inhibiting the downstream response[3]. In this study, we have analysed whether HCV NS3-4A is also able to cleave the peroxisomal MAVS and whether this would have any effect on the cellular antiviral response. We show that NS3-4A is indeed able to specifically cleave this protein and release it into the cytosol. Under these conditions, RIG-I-like receptor (RLR) signalling from peroxisomes is blocked and antiviral gene expression is inhibited. Our results also show that NS3-4A is able to localize at peroxisomes in the absence of MAVS. However, mutation studies have shown that this localization pattern is preferred in the presence of a fully cleavable MAVS. These findings present evidence of a viral evasion strategy that disrupts RLR signalling on peroxisomes and provide an excellent example of how a single viral evasion strategy can block innate immune signalling from different organelles.

HCV NS3-4A is able to specifically cleave the peroxisomal MAVS

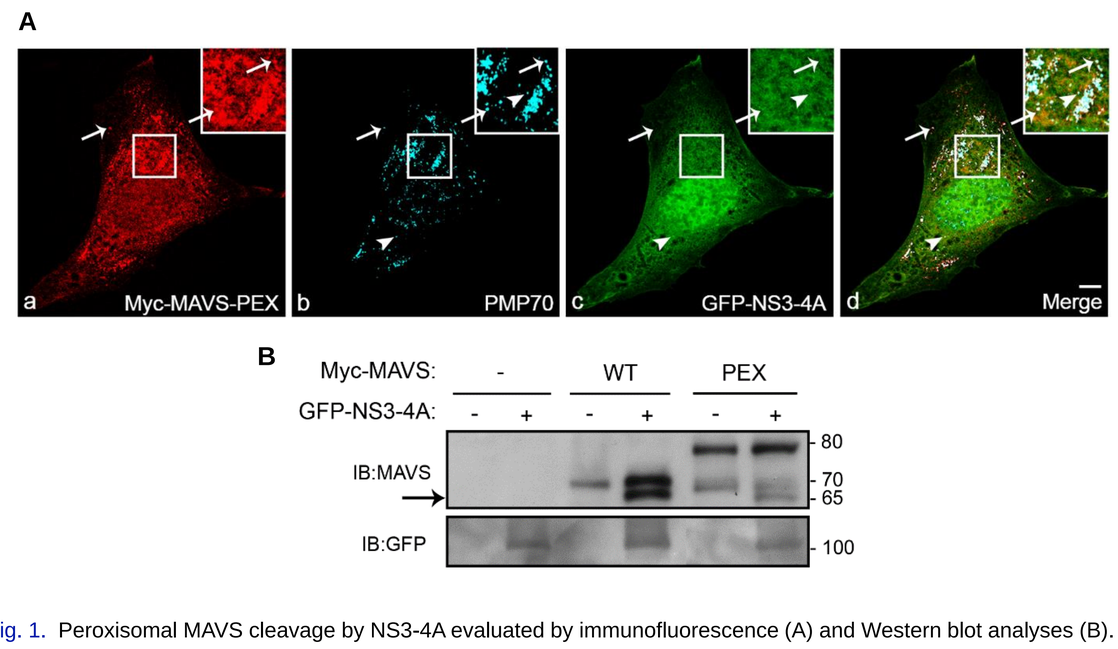


Fig. 1. Peroxisomal MAVS cleavage by NS3-4A evaluated by immunofluorescence (A) and Western blot analyses (B).

Mouse embryonic fibroblasts (Mefs) MAVS-KO cells were co-transfect with Myc-MAVS-PEX, a MAVS construct that localizes exclusively at peroxisomes, and GFP-NS3-4A. Cells were subjected to immunolocalization against the peroxisomal marker PMP70 and Myc-tag and observed by confocal microscopy. Arrows in Fig. 1A indicate co-localization between peroxisomes, MAVS-PEX and NS3-4A and full-head arrows indicate co-localization between peroxisomes and NS3-4A. As a control, for the Western blot analysis, we have also transfected Mefs MAVS-KO cells with Myc-MAVS-WT in the presence or absence of GFP-NS3-4A. Cells were analysed by immunoblot against MAVS and GFP. The arrow in Fig. 1B indicates the fragment produced by NS3-4A cleavage of MAVS.

These results show that peroxisomal MAVS is cleaved by NS3-4A, leading to the production of a fragment that is relocated to the cytosol.

NS3-4A cleavage of peroxisomal MAVS strongly inhibits the peroxisome-dependent antiviral cellular response

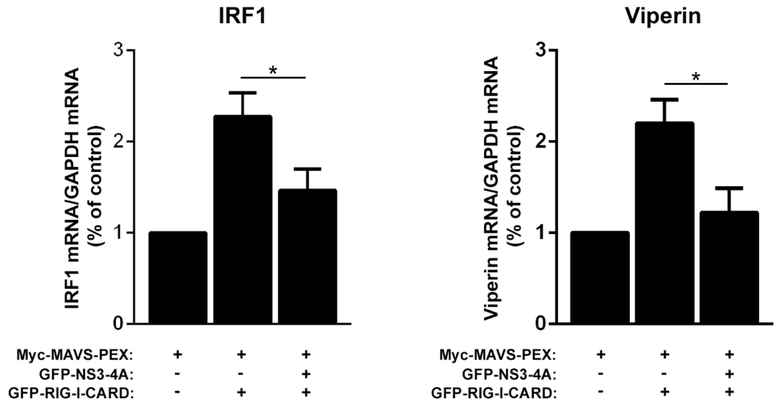


Fig. 3. Inhibition of peroxisomal MAVS downstream pathway by NS3-4A evaluated by RT-qPCR.

Mefs MAVS-KO cells were transfected with Myc-MAVS-PEX and stimulated with GFP-RIG-I-CARD in presence or absence of GFP-NS3-4A. Cell expression was analysed by RT-qPCR and the mRNA of the direct antiviral effectors IRF1 and viperin was quantified. GAPDH was used as control.

These results suggest that NS3-4A cleavage of MAVS-PEX disrupts the downstream pathway from peroxisomal MAVS limiting the expression of antiviral effector genes as IRF1 and viperin.

NS3-4A is able to traffic to peroxisomes in the absence of MAVS but preferentially targets this organelle in the presence of a fully cleavable version of this protein

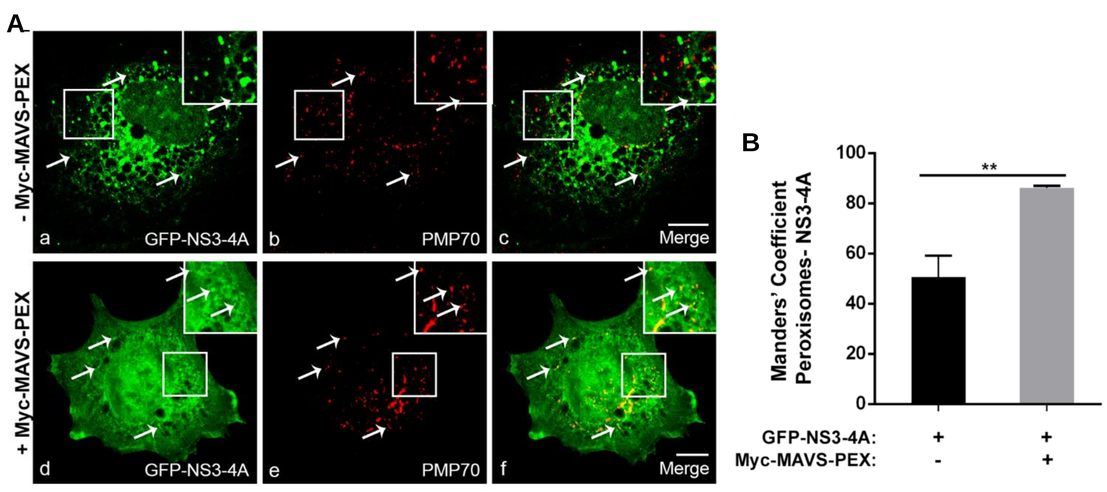
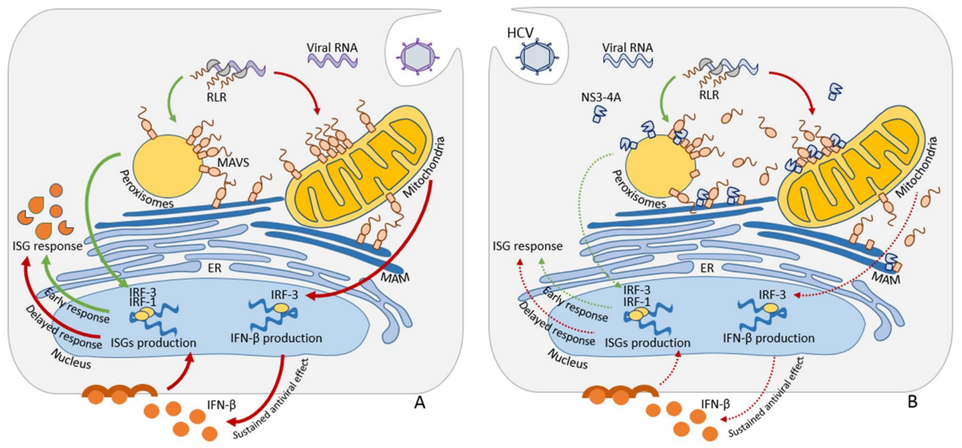


Fig. 4. NS3-4A intracellular localization evaluated by immunofluorescence (A) and co-localization analyses by Manders' coefficient (B).

Mefs MAVS-KO cells were transfected with GFP-NS3-4A in the presence or absence of Myc-MAVS-PEX. In Fig. 4, arrows indicate co-localization between NS3-4A and peroxisomes.

These results show that NS3-4A traffics to peroxisomes in the absence of MAVS but this attraction is stronger in the presence of this protein.

Conclusions



Hepatitis C virus NS3-4A targets MAVS present at peroxisomes disrupting the production of direct antiviral effectors.

Peroxisomes may be used as specific cellular targets for combat strategies against Hepatitis C virus.

References

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