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LETTERS

RNA interference screen for human genes associated with West Nile virus infection

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West Nile virus (WNV), and related flaviviruses such as tick-borne encephalitis, Japanese encephalitis, yellow fever and dengue viruses, constitute a significant global human health problem[1]. However, our understanding of the molecular interaction of such flaviviruses with mammalian host cells is limited[1]. WNV encodes only 10 proteins, implying that it may use many cellular proteins for infection[1]. WNV enters the cytoplasm through pH-dependent endocytosis, undergoes cycles of translation and replication, assembles progeny virions in association with endoplasmic reticulum, and exits along the secretory pathway[1–3]. RNA interference (RNAi) presents a powerful forward genetics approach to dissect virus–host cell interactions[4–6]. Here we report the identification of 305 host proteins that affect WNV infection, using a human-genome-wide RNAi screen. Functional clustering of the genes revealed a complex dependence of this virus on host cell physiology, requiring a wide variety of molecules and cellular pathways for successful infection. We further demonstrate a requirement for the ubiquitin ligase CBLL1 in WNV internalization, a post-entry role for the endoplasmic-reticulum-associated degradation pathway in viral infection, and the monocarboxylic acid transporter MCT4 as a viral replication resistance factor. By extending this study to dengue virus, we show that flaviviruses have both overlapping and unique interaction strategies with host cells. This study provides a comprehensive molecular portrait of WNV–human cell interactions that forms a model for understanding single plus-stranded RNA virus infection, and reveals potential antiviral targets.

The host proteins previously reported to facilitate WNV infection (termed host susceptibility factors, HSFs) comprise endosomal transport regulators and vATPase (for entry), eEF1A, TIA-1/TIAR and HMGCR (for replication), and c-Yes (for secretion)[2,3,7–10]. Other host proteins may reduce WNV infection (termed host resistance factors, HRFs): components of the antiviral IRF3 pathway are known HRFs of WNV infection[11]. In this context, we performed a genome-scale small interfering RNA (siRNA)-based screen silencing 21,121 human genes in HeLa cells to comprehensively identify the cellular proteins associated with the early stages of WNV infection, from viral entry through to the intracellular translation of viral RNA. Defects in the later stages of infection, such as replication, assembly or secretion, were not scored by the assay. The assay involved infection of gene-silenced cells with WNV for 24 h, followed by a microscopy-based quantification of the cells immunostained for viral envelope protein to select the candidate host proteins. The screen was done in two steps: a primary screen using a pool of four siRNAs per gene, followed by a validation screen, testing each individual siRNA within the pool separately (for the hits selected in the primary screen) to minimize potential off-target hits (Fig. 1a). The details of the assay and screen are described in Methods and Supplementary Fig. 1.

The RNAi screen identified 283 HSFs and 22 HRFs (of which 273 and 21 respectively are novel; Supplementary Tables 1 and 2). The number of HRFs constituted 7% of the total host factors identified. The identification of (1) some of the known HSFs (vATPase, endosomal transport regulators[3]) and HRFs (IRF3; ref. 11) of WNV infection, and (2) multiple components of macromolecular assemblies—for example, vATPase, the endoplasmic-reticulum-associated degradation (ERAD) pathway, focal adhesion complex (FAC)—validated the reliability of our approach and the *in vitro* model. A cellular map summarizing several screen hits classified into cellular compartments and broad functional association categories is provided in Supplementary Fig. 2.

Of the 283 HSFs, 195 (69%) and 193 (68%) could be classified using biological process and molecular function categories, respectively (Fig. 1b, c; Supplementary Tables 3 and 4). There was a significant enrichment of genes regulating intracellular protein trafficking, cell adhesion and processes associated with the transport of ions and biomolecules. The enriched molecular function categories included hydrolases, transporters, ligases, cell adhesion molecules, membrane traffic proteins and synthases. Among the HSFs, 6 RNA-binding proteins (for example, RBPMS), 20 ubiquitination-related proteins (for example, CBLL1), 21 transcription factors (for example, LDB1), 3 C-type lectins (CLEC7A, CLEC4A and CLEC4C) and 5 protocadherins (for example, PCDHB5) were also present. The RNA-binding protein RBPMS was reported as part of a protein network implicated in Purkinje cell degeneration[12]. Strikingly, the current screen also captured seven other members (COIL, PCP4, UBE2I, LDB1, NUMBL, ATXN7L3 and USP6) interacting with RBPMS (Supplementary Figs 3a, b, and 4a, b).

The screen also identified several genes previously implicated in immunity (Supplementary Tables 1 and 2). Immune related HSFs include β-defensins (DEFB118 and DEFB129, Supplementary Fig. 5a), Rnase L inhibitor ABCE1 (refs 13–15; Supplementary Fig. 5b), LY6E, Zap70, TNFSF13B and DUBA (OTUD5). Among the HRFs, α-defensin DEFA3 and IRF3 are known immune response genes. These findings highlight that defensin family members function as both viral resistance and susceptibility factors[16]. Knockdown of the immunophilin FKBP1B also enhanced WNV infection.

We next determined whether the genes identified from HeLa cells are expressed in tissues targeted by WNV *in vivo* by analysing the

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