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MHC class II transactivator CIITA induces cell resistance to Ebola virus and SARS-like coronaviruses

Anna Bruchez^{1*†}, Ky Sha^{1*}, Joshua Johnson^{2‡}, Li Chen³, Caroline Stefani¹, Hannah McConnell^{1§}, Lea Gaucherand^{1¶}, Rachel Prins¹, Kenneth A. Matreyek^{4†}, Adam J. Hume^{5,6}, Elke Mühlberger^{5,6}, Emmett V. Schmidt⁷, Gene G. Olinger^{2,5,6,8}, Lynda M. Stuart^{1,9}, Adam Lacy-Hulbert^{1,10#}

¹Benaroya Research Institute, Seattle, WA 98101, USA. ²National Institute of Allergy and Infectious Diseases (NIAID) Integrated Research Facility, Frederick, MD 21702, USA. ³Massachusetts General Hospital, Boston, MA 02114, USA. ⁴Department of Genome Sciences, University of Washington, Seattle, WA 98109, USA. ⁵Boston University School of Medicine, Boston, MA 02118, USA. ⁶National Emerging Infectious Diseases Laboratories, Boston University, Boston, MA 02118, USA. ⁷Merck and Co., Inc, Kenilworth, NJ 07033, USA. ⁸MRIGlobal, Gaithersburg, MD 20878, USA. ⁹Bill and Melinda Gates Foundation, Seattle, WA 98109, USA. ¹⁰Department of Immunology, University of Washington, Seattle, WA 98109, USA.

*These authors contributed equally to this work. †Present address: Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA. ‡Present address: AbViro LLC, Bethesda, MD 20814, USA. §Present address: Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA. ¶Present address: Graduate Program in Molecular Microbiology, Tufts Graduate School of Biomedical Sciences and Department of Molecular Biology and Microbiology, Tufts University, Boston, MA 02155, USA.

#Corresponding author. Email: alacyhulbert@benaroyaresearch.org

Recent outbreaks of Ebola virus (EBOV) and SARS-CoV-2 have exposed our limited therapeutic options and poor understanding of cellular mechanisms that block viral infections. Using a transposon-mediated gene-activation screen in human cells, we identify that the MHC class II transactivator (CIITA) has antiviral activity against EBOV. CIITA induces resistance by activating expression of the p41 isoform of invariant chain CD74, which inhibits viral entry by blocking cathepsin-mediated processing of the Ebola glycoprotein (EboGP). We further show that CD74 p41 can block the endosomal entry pathway of coronaviruses, including SARS-CoV-2. These data therefore implicate CIITA and CD74 in host defense against a range of viruses, and identify an additional function of these proteins beyond their canonical roles in antigen presentation.

Recent and ongoing outbreaks of Ebola virus (EBOV) in Africa (1) and the SARS-CoV-2 pandemic highlight the need to identify additional treatment strategies for viral infections, including approaches that might complement traditional antivirals. Of particular interest is the identification of host-directed therapies that target common vulnerabilities and may be potentially efficacious against multiple viruses, including those that may emerge in the future.

We set out to identify host pathways of cellular resistance to pathogens with pandemic potential, using a transposon-mutagenesis forward genetic approach. We used a modified PiggyBac (PB) transposon (Fig. 1A), which stimulates or disrupts expression of neighboring genes, allowing an interrogation of both gene activation and inactivation in a single screen (2). Transposon-mutagenized libraries were treated with Ebola glycoprotein (EboGP)-expressing recombinant vesicular stomatitis virus (referred to as EboGP-VSV). Susceptible wild-type (wt) U2OS cells died after 3–4 days of treatment, whereas surviving cells could be expanded from mutagenized libraries and exhibited stable resistance to re-challenge with EboGP-VSV (Fig. 1B). These cells showed no cross-resistance to VSV containing the VSV glycoprotein (VSVg-VSV) (Fig. 1C), suggesting that the majority of resistance mechanisms

selected in this screen targeted EboGP-mediated entry.

We identified candidate resistance genes by identifying genomic regions with high numbers of transposon insertions (“common insertion sites” or CISs) (3). Combining data from eight independent screens revealed seven genomic loci with highly statistically significant ($p < 10^{-8}$) CISs that occurred in more than one screen, representing high-confidence candidate-resistance mutations (Fig. 1D, outer ring). Likely target genes of transposon insertions were identified based on transposon insertion position and orientation (Fig. 1D and table S1). We focused on the two genes found in all eight screens using the most stringent criteria.

The first of these was *NPCI*, located on chromosome 18. All transposon insertions at this site were intragenic, in both sense and anti-sense orientations and were predicted to disrupt *NPCI* expression (Fig. 1E). This is consistent with the role of *NPCI* as the EBOV receptor (4, 5) and validates our screening approach. Notably, U2OS cells are haploid at the *NPCI* locus (6) and these transposon insertions are therefore predicted to generate *NPCI*-null cells, explaining why *NPCI* was the only predicted gene-disruption mutant identified as a high-stringency candidate gene.

All transposon insertions at the second CIS, on

chromosome 16, were upstream of the gene *CIITA*, and were all oriented in the sense orientation, consistent with activation of expression (Fig. 1F and fig. S1). *CIITA* overexpression in wt U2OS cells increased cell survival, reduced GFP reporter expression, and completely inhibited plaque formation, confirming that *CIITA* increases resistance to EboGP-VSV 100-1000-fold (Fig. 2, A to E, and fig. S2). *CIITA*-overexpressing cells were also resistant to EboGP-pseudotyped single cycle viruses (Fig. 2, F and G), strongly suggesting that *CIITA* inhibits viral entry rather than targeting viral transactivators as suggested for HIV and HTLV (7, 8). Furthermore, using EboGP-virus like particles (EboGP-VLPs) carrying β -lactamase (BLAM) (9), we found that *CIITA* did not affect the internalization of EboGP-VLPs into cells (Fig. 2H), but blocked viral fusion, which occurs in the endosome (10) (Fig. 2I). *CIITA*-expressing U2OS cells were also highly resistant to infection by high-titers of native EBOV, showing reduced reporter gene expression, cell death, and plaque formation (Fig. 2, J to M). *CIITA* expression did not inhibit replication of an EBOV minigenome, indicating that *CIITA* does not act on the viral replication complex (fig. S3). Furthermore, *CIITA* inhibited infection mediated by GPs from a range of EBOV species, including Sudan, Zaire, and Reston, as well as the distantly related filovirus Marburg virus (Fig. 2G). Thus, *CIITA* induces broad antiviral activity against EBOV and other pathogenic filoviruses through the inhibition of viral GP-mediated entry.

CIITA, also known as NLRA, is a Nod-like receptor (NLR) (11), but unlike most other NLRs, which function as cytosolic sensors, *CIITA* is a transcription factor (12). We therefore hypothesized that its antiviral activity occurred through the altered expression of host target genes. Supporting this hypothesis, mutation of domains required for transcriptional activity completely ablated *CIITA* antiviral activity (fig. S4). Resistance also required NF-Y, a component of the enhanceosome multi-protein complex, which mediates transcriptional activation by *CIITA* (13), but was independent of another enhanceosome protein, RFX5 (figs. S4 and S5). Antiviral activity was therefore mediated by a subset of NF-Y-dependent RFX5-independent *CIITA* target genes, which include genes associated with antiviral immunity (14). Systematic knock-down of all *CIITA* target genes identified a single gene, *CD74*, required for *CIITA*-mediated resistance (Fig. 3, A and B). This was confirmed by CRISPR knockout (ko) of *CD74* expression and function in *CIITA*-overexpressing cells (Fig. 3C).

Both *CIITA* and *CD74* are expressed at high levels by macrophages and dendritic cells (DCs), which are early targets of EBOV (15, 16). To test whether *CIITA* has antiviral activity in immune cells, we used primary bone marrow-derived macrophages (BMDMs) from *Ciita*^{-/-} and *Cd74*^{-/-} mice. Naïve BMDMs did not express high levels of *CIITA* or *CD74*, and showed no difference in viral fusion. Treatment with IFN- γ

and LPS induced expression of *CIITA* and *CD74*, and IFN- γ /LPS primed *Ciita*^{-/-} and *Cd74*^{-/-} BMDMs had higher levels of EboGP-VLP fusion than in equivalent wt cells (Fig. 3, D to G, and fig. S6). Similar results were seen in *Cd74*^{-/-} bone marrow-derived DCs and in a *CD74*^{-/-} human macrophage-like cell line (differentiated THP-1) (figs. S7 and S8). Thus, endogenous *CIITA* and *CD74* have antiviral activity in primary immune cells, which can be induced by exposure to IFN- γ and LPS.

CD74 is the MHC-II invariant chain and human cells express four main isoforms of *CD74*, differing by the presence of a N-terminal ER-retention signal and an internal thyroglobulin domain (Fig. 4A) (17). Only one *CD74* isoform, p41, was able to fully rescue resistance to EboGP-VSV infection in *CIITA*-expressing, *CD74*-knockout cells (Fig. 4B and fig. S9). p41 conferred resistance independently of *CIITA* expression (Fig. 4C), demonstrating that *CD74* p41 expression was sufficient to induce antiviral activity. This property of *CD74* was not limited to U2OS cells, as *CD74* p41 similarly inhibited fusion when expressed in THP-1 cells (Fig. 4D). The p41 isoform contains the thyroglobulin domain, lacks the ER retention signal, and normally accumulates in endosomes. Mutant constructs of *CD74* revealed that only the thyroglobulin domain is essential for antiviral activity, but dissociation from the membrane, either by addition of a furin cleavage site (“furin”) or deletion of the transmembrane sequence (“No TM”), or delivery to the cell surface, by fusion to a heterologous cytoplasmic and transmembrane sequence from tetherin (“tetherin”; Fig. 4E) almost completely removed antiviral activity (Fig. 4E and fig. S10). Thus, antiviral activity required delivery of the thyroglobulin domain to the endosomal membrane. Electron microscopy showed that EboGP-VSV virions accumulated in late endosomal multivesicular bodies (MVBs) of *CIITA*- and *CD74*p41-expressing cells, with some virions within intraluminal vesicles (Fig. 4F and fig. S11). Confocal microscopy confirmed that VLPs localized proximal to CD63 and the ESCRT component Hrs which mark MVBs (18, 19) (Fig. 4, G and H). Thus, *CIITA* and *CD74* p41 inhibit fusion by arresting viral particles in MVB compartments.

EBOV entry requires endosomal cathepsins (4, 10, 20) (fig. S12), which sequentially process EboGP (Fig. 4I and fig. S13). The *CD74* thyroglobulin domain inhibits cathepsins (21), suggesting that this may be the mechanism for antiviral activity. In support of this, *CD74* inhibited EboGP processing, similar to the effects of the cathepsin L (CTSL) inhibitor FYDMK (Fig. 4I). Additionally, disruption of the p41 CTSL binding site (22, 23) by mutation completely inhibited antiviral activity (Fig. 4J and fig. S10). Glycoprotein cleavage by endosomal proteases is critical for the entry of other viruses, including coronaviruses. SARS-CoV and SARS-CoV-2 S proteins can be processed by either endosomal cathepsin B and L, or alternatively by cell-surface serine proteases including TMPRSS2

(24, 25). In TMPRSS-expressing cells, such as lung epithelium, inhibition of both cathepsins and serine proteases is required to inhibit viral entry, whereas cathepsin inhibitors alone block infection in cell lines, such as U2OS and Vero cells, that lack TMPRSS2 (25). p41 inhibited entry of viruses pseudotyped with S proteins from SARS-CoV and a related bat virus WIV1-CoV into U2OS cells, demonstrating that p41 inhibited S protein processing (Fig. 4K). To determine whether p41 exhibited antiviral activity against authentic SARS coronavirus, we challenged p41-expressing Vero E6 cells with SARS-CoV-2. CD74 p41 expression completely inhibited plaque formation, demonstrating that this antiviral activity extended beyond filoviruses (Fig. 4L).

In summary, we identify the antiviral activity of CIITA and CD74. We show that CIITA induces resistance by up-regulation of the p41 isoform of CD74, which blocks cathepsin-mediated cleavage of viral glycoproteins, preventing viral fusion. This antiviral activity protects against a wide range of cathepsin-dependent viruses, including filoviruses and coronaviruses, functions in macrophages and DCs that are early targets of infection (15, 16), and is activated by IFN- γ . We demonstrate that CIITA and CD74 mediate the endosomal sequestration of certain viruses as a mechanism of cellular host defense. We speculate that this activity has been co-opted through evolution to facilitate antigen processing and preceded their better-known role in antigen processing, a process that sits at the intersection of innate and adaptive immunity. We anticipate that the application of this transposon screening approach to other models of infection will reveal other mechanisms that have eluded conventional screening strategies.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

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Fig. 1. Transposon-mediated activation-tagging generates mutant cells resistant to Ebola. (A) Modified PiggyBac (PB) transposon. (B and C) Resistance of selected cells to EboGP-VSV (B), and VSVg-VSV (C). Mean $\pm$ SD of $n=3$ replicates for one representative pool. Student's t test, $**P < 0.01$. (D) Distribution of transposon insertions. Inner rings show insertions per 1Mb for individual libraries (black histograms) and common insertion sites ($P < 10^{-7}$). Outer ring shows combined insertions for all libraries (black histogram) and lowest P -value for common insertion sites (red bubble plot). Point size represents number of libraries with common insertion site. (E and F) Cumulative independent insertions from all eight libraries mapping to *NPC1* and *CIITA*.

Fig. 2. Identification of CIITA as an Ebola restriction factor. (A) Resistance of CIITA-overexpressing and control U2OS cells to EboGP-VSV. (B and C) Plaque formation assays (B) and effective viral titer (C) for control and CIITA-overexpressing U2OS cells infected with VSVg-VSV (VSV) and EboGP-VSV (Ebo). (D) Representative images of CIITA-transfected (CIITA), control-transfected (Cntrl), and unmanipulated U2OS cells (U2OS) infected with mCherry-expressing EboGP-VSV (red) and stained with Hoechst 33342 to resolve cell nuclei (blue). (E to G) Infection of control and CIITA-expressing U2OS cells by EboGP-VSV, LFVGP-VSV, or VSVg-VSV (E), VSVg-MLV and EboGP-MLV (F), or HIV pseudotyped with VSVg or GP from EBOV, Tai Forest virus (TAFV), Bundibugyo virus (BDBV), Sudan virus (SUDV), Reston virus (RESTV), or Marburg virus (MARV) (G). (H and I) Internalization (H) and fusion (I) of EboGP-VLPs by control and CIITA-overexpressing U2OS cells. No env: non-enveloped control VLPs. (J to M) Infection of control and CIITA-overexpressing U2OS cells by infectious Ebolavirus (EBOV) measured by imaging of GFP reporter (green) and cell nuclei (blue) (J), cell survival (K), infected cells (L) or plaque formation (M). Data are means $\pm$ SEM of three independent experiments (A-I) or experiments with three independent cell clones (K-M). Student's *t* test (A,C,K-M) or ANOVA with Tukey's multiple comparison test (E-I); **P* < 0.05; ***P* < 0.01. ND: not detected). Scale bars: 100 μ m.

Fig. 3. Transcriptional activity of CIITA and enhanceosome components are required for resistance. (A) Genes regulated by CIITA in U2OS cells, with strongest induced genes identified. Mean of three independent CIITA-expressing clones and controls. (B) EboGP-VSV infection of CIITA-expressing cells treated with siRNA against CIITA transcriptional targets. Data from two siRNAs per gene, $N=3$ independent screens, bars indicate mean with 95% CI. One-way ANOVA with Bonferroni's multiple comparisons; $*P<0.05$ and $**P<0.01$. Dotted lines indicate 99% CI from no siRNA control. (C) CD74 CRISPR-targeting in CIITA-overexpressing U2OS cells was verified by immunoblot, and infection and survival measured following EboGP-VSV challenge. Means $\pm$ SEM of $N=3$ experiments using two independent cell clones. (D and E) *Ciita* and *Cd74* expression in wt or *Ciita* $^{-/-}$ mouse BMDMs with or without priming by IFN- γ and LPS. (F and G) Fusion of EboGP-VLPs in unprimed (F) or primed (G) mouse BMDM from *Ciita* $^{-/-}$ and *Cd74* $^{-/-}$ mice, measured as geometric mean fluorescence (GMFI) of cleaved CCF2. Means $\pm$ SEM for independent cultures from three mice/group. Student's t test; $*P<0.05$; $**P<0.01$. Similar results were observed in three independent experiments.

Fig. 4. CD74 p41 inhibits cathepsin-mediated cleavage of EboGP. (A) Human CD74 isoforms with ER-retention signal (ER), CLIP, acidic, and p41 thyroglobulin domains. (B and C) EboGP-VSV infection and survival of *Cd74*^{-/-} CIITA-expressing (B) or wt (C) U2OS cells expressing CD74 isoforms. (D) EboGP-VLP fusion in THP-1 macrophage like cells expressing CD74 p33 and p41. (E) EboGP-VSV infection of U2OS cells expressing CD74 mutant constructs. (F) Transmission electron micrographs of control, CIITA-expressing, and CD74-expressing U2OS cells, 3 hours after infection with EboGP-VSV. Dotted line regions enlarged in adjacent panels. Intraluminal vesicles (arrowheads) and internalized EboGP-VSV (arrows) are marked. Scale bars: 1 μ m (left, center-left, and right panels), 200 nm (center-right panels). (G) Confocal microscopy of control and p41-expressing U2OS cells showing EBOV-VLP (red), CD63, or Hrs (green), and nuclei (white). Scale bars: 10 μ m. (H) VLPs associated with CD63 endosomes in U2OS cells expressing CIITA and CD74 as indicated. Each point represents a single cell, mean $\pm$ SD $n \geq 9$. Mann-Whitney *U* test; $**P < 0.01$. Similar results seen in three independent experiments. (I) Immunoblot of EboGP in EboGP-VSV-infected U2OS cells. EboGP-VSV preparation $\pm$ thermolysin (Therm) shown for reference (left). Cells were treated with cathepsin inhibitors E64D (E) or FYDMK (F), or expressed CIITA and CD74. EboGP in virus particles (arrow), after proteolysis (closed arrowhead) and after partial cleavage (open arrowhead) are indicated. (J) EboGP-VSV infection of U2OS cells expressing p41 with CTSL binding site mutations. (K) Infection of control, p33-, or p41-expressing U2OS cells by HIV-GFP pseudotyped with glycoproteins from VSV, EBOV, SARS-CoV, or WIV1-CoV, measured as focus forming units per milliliter of virus (FFU/ml). (L) Infection of control, p33-, or p41-expressing Vero cells by SARS-CoV-2, showing representative crystal violet-stained monolayers and infection measured as plaque-forming units per milliliter of virus (PFU/ml). Except where indicated, data are mean $\pm$ SEM of data from ≥ 3 independent experiments. Student's *t* test with Benjamini correction; $*P < 0.05$; $**P < 0.01$.