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Non-coding RNAs and heme oxygenase-1 in vaccinia virus infection

Clement A. Meseda [b], Kumar Srinivasan [a,†], Jasen Wise [c], Jennifer Catalano [d], Kenneth M. Yamada [e], Subhash Dhawan [a,*]

[a] Division of Transfusion Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, United States
[b] Division of Viral Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, United States
[c] Qiagen, Frederick, MD, United States
[d] Center for Tobacco Products, Food and Drug Administration, Bethesda, MD, United States
[e] National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, United States

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ABSTRACT

Small nuclear RNAs (snRNAs) are <200 nucleotide non-coding uridylate-rich RNAs. Although the functions of many snRNAs remain undetermined, a population of snRNAs is produced during the early phase of infection of cells by vaccinia virus. In the present study, we demonstrate a direct correlation between expression of the cytoprotective enzyme heme oxygenase-1 (HO-1), suppression of selective snRNA expression, and inhibition of vaccinia virus infection of macrophages. Hemin induced HO-1 expression, completely reversed virus-induced host snRNA expression, and suppressed vaccinia virus infection. This involvement of specific virus-induced snRNAs and associated gene clusters suggests a novel HO-1-dependent host-defense pathway in poxvirus infection.

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1. Introduction

Although immunization is one of the most cost-effective health interventions, mass-scale vaccination may not be feasible for a sudden outbreak of poxvirus disease such as smallpox or monkeypox. Moreover, there is little choice of conventional antiviral drugs to treat poxvirus-related complications. Induction of an innate host protective response could provide a potentially novel, effective, and safe therapeutic strategy for the treatment of poxvirus infections.

Because host factors can contribute significantly to productive virus replication in infected cells, altering virus–host interactions could interrupt virus replication cycles in infected cells and protect against pathogenic infection. We have previously shown that heme oxygenase-1 (HO-1), a pivotal cytoprotective gene, can be induced to inhibit HIV-1, West Nile virus, dengue, and *Leishmania donovani* infection of human monocyte-derived macrophages (MDM) [1–5]. HO-1 induction has also been reported to suppress hepatitis B virus, hepatitis C virus, and malaria infections [6–10], Recently, Hill-Batorski et al. reported HO-1-dependent suppression of Ebola virus replication [11], further establishing a key role for this endogenous enzyme in host defense.

In the present study, we demonstrate that modulation of HO-1 reduces virus yield with dramatic regulation of virus-induced non-coding RNAs during host defense against vaccinia infection. To our knowledge, our studies are the first to identify the coordinated induction of small non-coding RNAs in vaccinia virus infection, with suppression of a host-defense strategy. Blocking vaccinia-induced snRNA could be a potential target for therapeutic intervention against viral infection.

2. Materials and methods

The FDA-approved drug Panhematin® was purchased from Lundbeck, Deerfield, IL (manufactured by APP Pharmaceuticals, Raleigh, NC). Human MDM were generated from normal donors [7] and infected with vaccinia virus strain Western Reserve (VV-WR), or other strains of vaccinia virus as described previously [12]. Virus replication was quantified by plaque assay using BSC-40 cells [13]. HO-1 induction in MDM was determined by ELISA (Enzo Life Sciences, Farmingdale, NY). Cellular toxicity was assessed by trypan blue exclusion tests of untreated and hemin-treated MDM plated in 24-well cell culture plates. Wright-staining of uninfected, vaccinia-infected and hemin-treated vaccinia-infected MDM was performed, and the stained cells in high power field (HPF) were scored by light microscopy. Whole-genome expression of reverse-transcribed RNA from uninfected and vaccinia-infected MDM cultured in the presence or absence or hemin was performed using the Illumina HT12_V4 BeadChip Assay System.

* Corresponding author.
E-mail address: subhash.dhawan@fda.hhs.gov (S. Dhawan).
† Deceased.

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3. Results and discussion

Hemin treatment of MDM induced HO-1 in a dose-dependent manner (Fig. 1A), and it was markedly inversely correlated with decreased virus titer (Fig. 1B). MDM infected with a recombinant vaccinia virus WR strain expressing GFP showed a majority of cells expressing GFP (Fig. 1C), indicating effective virus infection. Infection was substantially reduced in cells treated with 150 μM hemin (Fig. 1C). Hemin treatment continued to suppress vaccinia infection of MDM at even higher multiplicity of infection (MOI) (Fig. 1D) or when challenged with other strains of vaccinia virus (data not shown). This hemin-induced cellular protection against

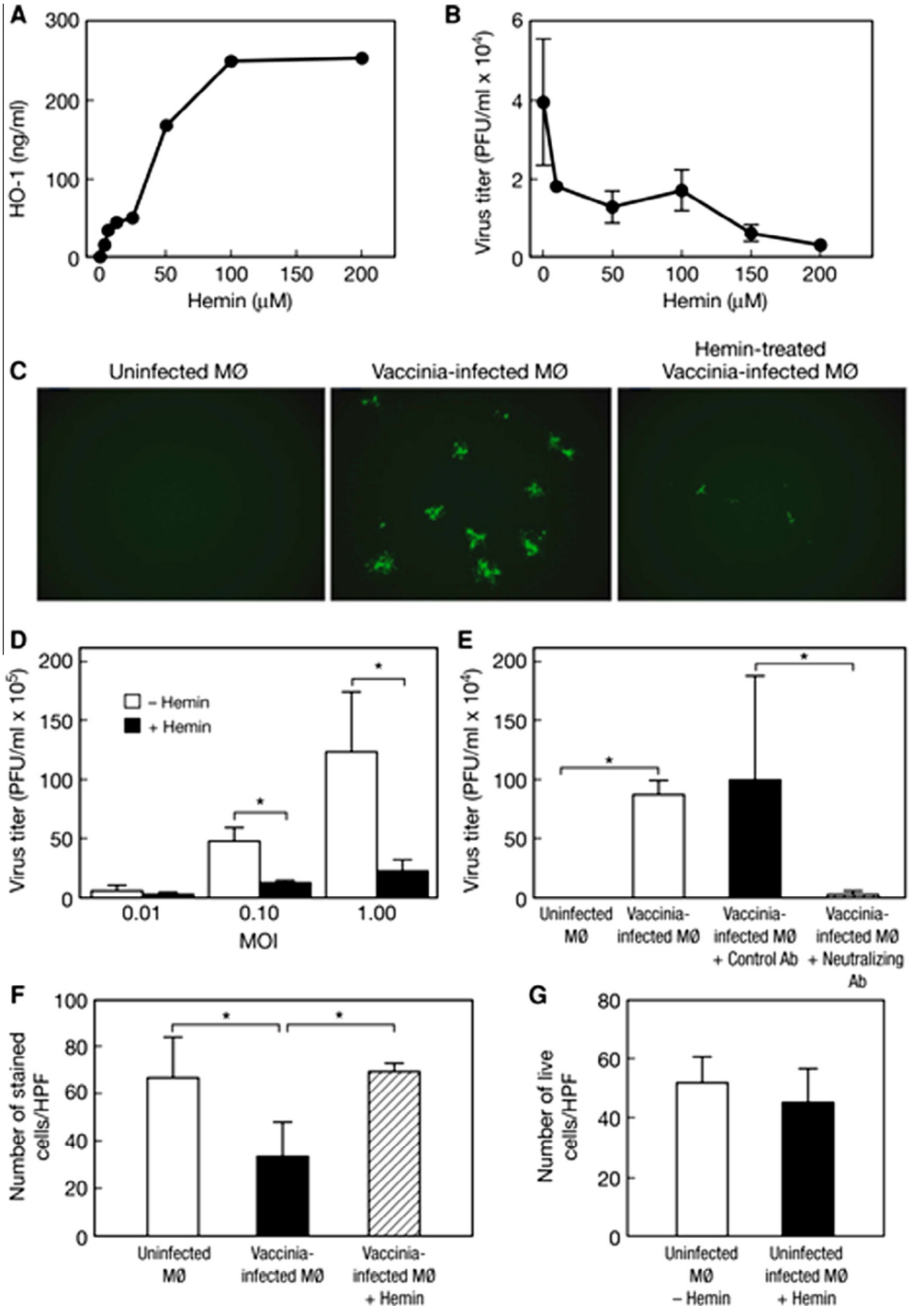

Fig. 1. HO-1 induction and inhibition of MDM vaccinia virus infection. (A) Representative data demonstrating HO-1 induction in MDM treated for 24 h with various concentrations of hemin. (B) Viral titer in VV-WR-infected MDM cultured for 48 h with the indicated concentrations of hemin. (C) GFP expression in MDM infected with GFP-tagged recombinant VV-WR in the absence or presence of 100 μM hemin. (D) Viral titer in MDM infected with vaccinia at various MOI in the absence or presence of 150 μM hemin. (E) Viral titer in MDM infected in the absence or presence of anti-L-1 neutralizing mAb (10F5). (F) Viability of uninfected, vaccinia-infected, and hemin-treated vaccinia-infected MDM cultured for 48 h in the absence or presence of vaccinia-neutralizing 10F5 mAb (mean ± SD). (G) Effect of hemin on the viability of MDM measured as the number of Wright-stained cells/HPF. Data are presented as mean ± SD determined microscopically after 48 h of hemin treatment. $^{*}p < 0.01$.

Table 1
HO-1 induction inhibits vaccinia-regulated host genes.

Symbol	Vaccinia-infected MØ vs. uninfected MØ		Hemin-treated vaccinia infected MØ vs. infected MØ	
	MOI = 0.01	MOI = 5	MOI = 0.01	MOI = 0.01
SNORD46	3.68	6.14	−3.18	−1.02
RNY1	36.90	15.25	−7.30	−1.63
RNU6-15	37.52	31.13	−5.46	−1.55
RNU1G2	82.67	194.97	−5.25	−2.34
SNORA7B	3.29	6.50	−2.65	−1.11
HIST1H4E	10.73	8.64	−5.68	−1.83
SCARNA2	27.44	49.62	−5.74	−1.14
RN5S9	176.76	225.41	−4.88	1.27
LOC100132564	34.17	128.39	−2.33	1.10
RNU6-1	35.54	29.68	−5.05	−1.49
SNORD3D	8.92	19.82	−4.76	1.82
RNU1F1	31.35	36.30	−6.40	−2.24
RNU1-3	74.45	240.85	−4.72	−2.66
VTRNA1-1	23.08	38.27	−7.75	−1.51
RNU105A	4.65	3.87	−2.96	1.44
RPL10L	3.94	3.29	−3.13	−1.23
LOC85389	19.89	16.99	−5.88	1.07
LOC100008589	28.65	156.60	−5.27	1.29
LOC100008589	25.87	118.58	−1.95	1.12
SNORD83B	5.83	11.40	−3.20	1.61
LOC100132394	30.39	134.88	−5.19	1.06
KREMEN2	3.74	20.53	−2.64	−2.00
SNORA73B	4.47	4.04	−3.01	1.25
SNORD15B	3.44	5.43	−2.65	1.23
KIAA1666	3.27	14.49	−2.47	−1.16
RNU4-2	12.13	106.99	−7.26	−2.26
RMRP	3.77	21.54	−3.46	−1.36
RNU1-5	54.26	176.62	−3.91	−2.23
LOC100008588	34.59	61.54	−15.91	−1.02
SNORA63	5.70	11.60	−4.30	−1.22
LOC100134364	22.59	80.80	−4.65	−1.26
ALB	6.41	5.98	−3.51	−1.55
HMOX1	−1.35	−1.28	**12.48**	**3.32**
HIST1H2BG	4.20	7.02	−3.82	−1.94
TRK1	6.35	70.74	−4.39	1.01
LOC441763	14.49	240.48	−12.47	−1.71
RNY5	4.97	10.96	−4.18	1.07
RNU4-1	14.25	161.21	−7.77	−2.21
SNORD3C	5.33	13.62	−3.50	1.85
HIST1H4F	4.19	5.47	−3.30	−1.82
LOC100133565	34.38	441.60	−24.16	−1.42
RPPH1	11.99	80.30	−6.71	−2.11
TMEM107	6.39	7.83	−4.67	−2.15
SNORA73A	4.16	4.18	−2.92	1.44
TXNIP	2.68	3.61	−4.08	−7.31
RN7SK	20.64	77.45	−2.87	−1.18
RN7SK	34.65	145.57	−3.88	−1.12
HIST2H4A	6.56	3.24	−4.89	−1.44
RNY4	42.60	28.78	−5.38	−1.63
HIST4H4	5.07	8.91	−4.38	−2.51
HIST1H4H	13.39	13.63	−4.84	−1.77
SNORD3A	6.73	19.06	−5.04	−1.78
RNU6ATAC	10.59	16.75	−7.60	−1.05

vaccinia virus, measured as the virus titer in the infected MDM, was similar to that provided by treatment with the vaccinia-neutralizing 10F5 monoclonal antibody [14] (Fig. 1E).

Vaccinia infection resulted in a substantial loss of MDM when compared with the uninfected control group as revealed by the reduced number of Wright-stained cells per high-power microscopic field (Fig. 1F). This loss of cells was prevented when MDM were pre-treated with hemin prior to infection with vaccinia virus (Fig. 1F). Possible hemin-induced cytotoxicity was excluded by counting the number of Wright-stained MDM present per high-power field in the absence of vaccinia infection. As shown in Fig. 1G, hemin treatment did not significantly reduce the number of MDM cultured for 48 h in the presence of 150 μM hemin, thus ruling out the possibility that the lower viral titer in hemin-treated cells was due to hemin-induced cytotoxicity. Taken together, these data suggest the potential for therapeutic use of hemin in treating vaccinia and possibly other poxvirus infections in unforeseen crisis situations when mass-scale immunization is not feasible, especially because no safe and effective drug is licensed for treating vaccinia or other poxvirus infections.

On order to identify potential pathways by which HO-1 or HO-1-related genes directly, indirectly, or cooperatively contribute to the inhibition of vaccinia infection of MDM, whole-genome expression analyses were performed on reverse-transcribed RNA isolated from cells 48 h after infection by microarray technology using Illumina human HT12_V4 chips with more than 47,000 probes. Primary MDM were cultured for 24 h in the absence or presence of 150 μM hemin and then infected with the VV-WR at MOI 0.01 and 5.0. Table 1 shows the induction of 54 genes in MDM infected VV-WR both at low and high MOI. VV-WR infection significantly increased expression of small nucleolar RNAs, small Cajal-specific RNA2, histone 1 and 4 gene clusters, and other critical host genes.

Fig. 2 depicts the heatmap profiles of vaccinia virus-induced host genes in MDM and their complete reversal by hemin treatment, associated with the expected increased expression of HO-1. Consistent with the reduction of virus titer by the 10F5 mAb (Fig. 1E), vaccinia-induced increased expression of these genes was completely blocked by the 10F5 mAb (data not shown)

A population of small nontranslated polyadenylylated RNAs is produced in vaccinia virus infection [15]. Fig. 3A shows the expression of ncRNAs substantially upregulated in vaccinia-infected cells (blue bars); remarkably, all were completely blocked with HO-1 induction (red bars). Fig. 3B depicts Ingenuity Pathway Analysis of the identified small ncRNAs, plus functionally linked genes. The significant genes listed in Table 1 were imported into the Ingenuity database, and the top scoring network is shown in panel a, which focuses on genes upregulated by vaccinia infection of MDM. Panel b shows the genes regulated by hemin induction in vaccinia-infected MDM. Accompanying the induction of Hmox1

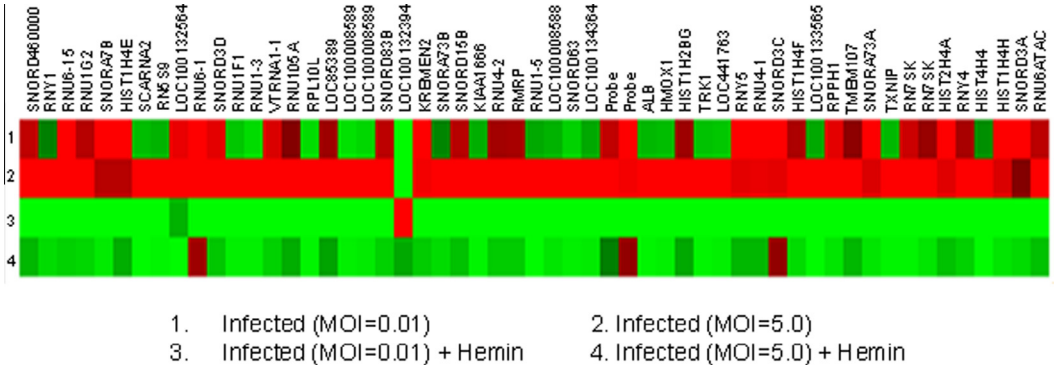

Fig. 2. Hemin HO-1-induction blocks vaccinia-induced gene expression in MDM. Comparison of heatmaps of host genes expressed in untreated and hemin-treated vaccinia-infected MDM.

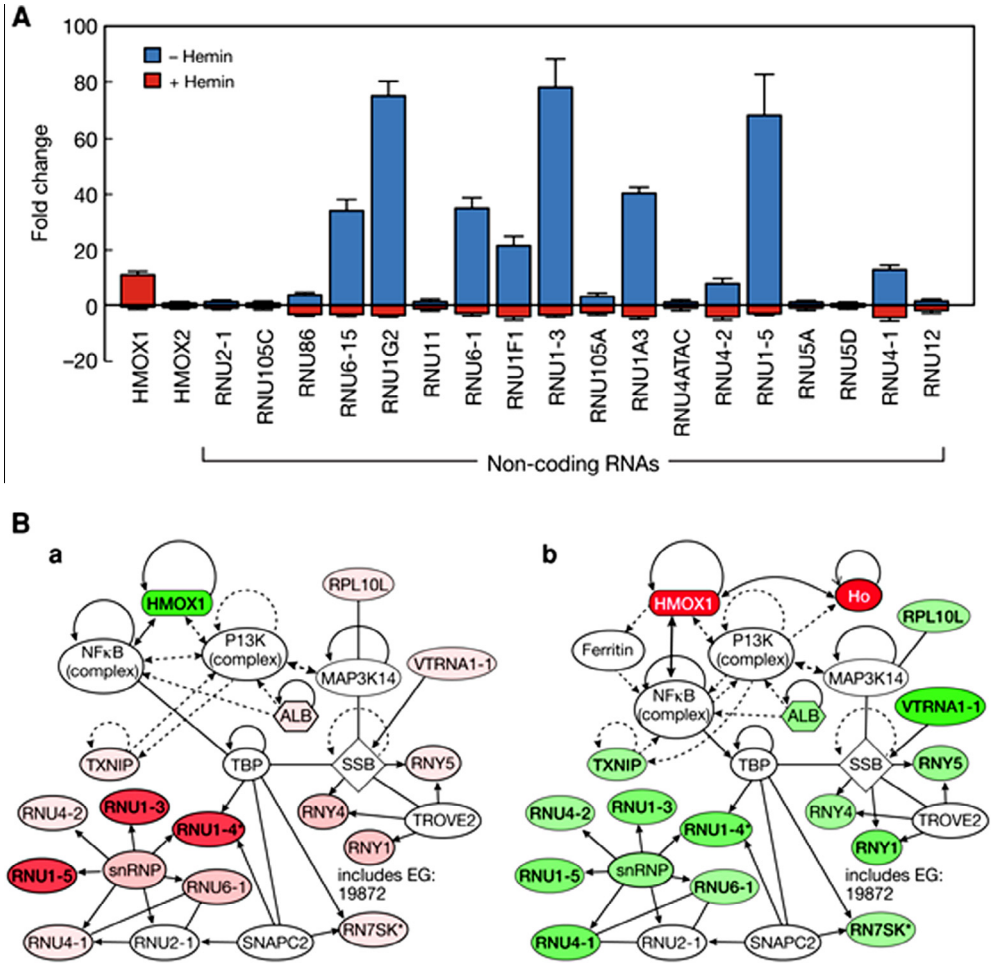

Fig. 3. (A) Inverse correlation between HO-1 induction and ncRNA expression in vaccinia-infected MDM. (B) Ingenuity Pathway Analysis of vaccinia-induced genes (a), and reversal with HO-1 induction (b). Red colored shapes indicate upregulation, green colored shapes indicate downmodulation of the regulated genes, and no change is indicated by clear symbols.

expression by hemin treatment of vaccinia-infected MDM, the upregulation of all other entities was completely reversed as shown by the green symbols in panel b.

While non-protein-coding RNAs represent the majority of human transcripts compared to protein-coding RNAs, their functions have generally remained mysterious. Our findings on selective snRNA regulation should provide useful information and hypotheses to help elucidate the mechanisms of HO-1-dependent transcriptional regulation in host defense against vaccinia virus infection. There is much to learn about the specific roles of these ncRNAs in normal human physiology and pathophysiology; our discovery of tightly regulated expression of this class of ncRNAs in vaccinia-infected and treated cells identifies a new pathway of virus–host interaction, thus expanding our knowledge about the potential regulation of small non-coding RNAs in disease pathogenesis and development of strategies for therapeutic intervention against poxvirus infection.

In summary, induction of the endogenous cytoprotective enzyme, HO-1, significantly inhibited the susceptibility of MDM to vaccinia infection, markedly increased cell viability, and ablated virus-induced host snRNA gene expression. Blocking of virus-induced snRNAs and related gene clusters may provide genomic pathways for HO-1-dependent host defense against poxvirus infection.

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