Arch Virol (2012) 157:1719–1727
DOI 10.1007/s00705-012-1366-z

ORIGINAL ARTICLE

Vaccinia virus infection suppresses the cell microRNA machinery

Moran Grinberg · Shlomit Gilad · Eti Meiri · Asaf Levy · Ofer Isakov · Roy Ronen · Noam Shomron · Zvi Bentwich · Yonat Shemer-Avni

Received: 10 November 2011 / Accepted: 27 April 2012 / Published online: 7 June 2012

Abstract MicroRNAs are key players in the regulation of gene expression by posttranscriptional suppression. They are involved in physiological processes, and thus their deregulation may contribute to the development of diseases and progression of cancer. Virus-encoded microRNAs and microRNAs of host origin play an important role in controlling the virus life cycle and immunity. The aim of this study was to determine the effect of vaccinia virus (VACV) infection on the expression of host-encoded microRNAs. A marked general suppression of most microRNAs in the infected cells was observed within 24 hours after VACV infection of a number of cell types. We demonstrate that this suppression was associated with abrogation of expression of the Dicer1 enzyme, which is a key enzyme in the generation of microRNAs.

Introduction

Vaccinia virus (VACV) belongs to the family *Poxviridae*, genus *Orthopoxvirus*. Members of this family have a large complex virion that contains a linear double-stranded DNA and mRNA synthesis enzymes [1]. Poxviruses are exceptional among DNA viruses, as they replicate in the cytoplasm within distinct cytoplasmic factories (CFs), where RNA transcripts, protein synthesis and DNA replication are coupled [2, 3]. Transcription, followed by protein synthesis, is characterized by three phases: early, intermediate and late. VACV competes with the host translation machinery and guarantees viral proteins synthesis through enhanced degradation of host mRNAs and sequestering crucial translation initiation factors within the cytoplasm to the VACV-CFs [2]. The enhanced mRNAs turnover is assisted by two decapping enzymes encoded by the virus, D9 and D10. Both enzymes contain a Nudix hydrolase motif and show 25 % sequence similarity [4, 5]. D9 is an early protein, while D10 is an intermediate-late protein.

As a consequence of readthrough transcription, at the intermediate and late phases of infection, double-stranded RNAs (dsRNA) are formed that are confined to the CF [6]. These double-stranded transcripts can activate interferon (IFN)-dependent resistance of the host to infection. Furthermore, they can also serve as substrates for Dicer1, generating small inhibitory RNAs (siRNA) that may lead to silencing of VACV genes. However, VACV is relatively resistant to host cell's protection mechanisms. The counter-activity against IFN is mediated through the expression of viral proteins such as B8R, an IFN-γ soluble receptor analogue; K3L, an eIF-2α homolog; or E3L, a dsRNA-binding protein. The last two abrogate the double-stranded RNA-dependent protein kinase (PKR) pathway that leads to inhibition of protein synthesis [7–11]. The way VACV copes with either siRNA or microRNA (miRNA) biogenesis has not been studied.

miRNAs are key regulators of gene expression by posttranscriptional suppression, a process that is highly conserved throughout evolution. They are involved in

Electronic supplementary material The online version of this article (doi:10.1007/s00705-012-1366-z) contains supplementary material, which is available to authorized users.

M. Grinberg · Z. Bentwich · Y. Shemer-Avni (✉)
Department of Virology and Molecular Genetics,
Faculty of Health Sciences, Ben Gurion University of the Negev,
84105 Beer Sheva, Israel
e-mail: yonat@bgu.ac.il

S. Gilad · E. Meiri · A. Levy
Rosetta Genomics Ltd., 10 Plaut St., 76706 Rehovot, Israel

O. Isakov · R. Ronen · N. Shomron
Cell and Developmental Biology, Faculty of Medicine,
Tel-Aviv University, Ramat-Aviv, Israel

almost every biological pathway, including developmental timing, cell differentiation, cell proliferation, cell death, metabolic control, transposon silencing and antiviral defense [12–14]. Transcription of miRNAs is typically performed by RNA polymerase II. The primary capped and polyadenylated transcripts (pri-miRNA) [15] are processed in the nucleus by Drosha to one or more precursors of microRNAs (pre-miRNA), which are then further processed in the cytoplasm by Dicer1 to form the mature miRNAs [15, 16]. miRNAs and siRNAs are incorporated into a ribonucleoprotein complex known as the RNA-induced silencing complex (RISC), and they direct the RISC to downregulate gene expression by either of two posttranscriptional mechanisms: mRNA cleavage or translational repression [17, 18].

Viruses are known to adopt several of the host's biological control mechanisms, using them to their own advantage. Hence, virus-encoded miRNAs could potentially control various phases of the viral life cycle, such as latency, reactivation, replication, etc. It is therefore not surprising that many DNA viruses, including herpesviruses, adenovirus, polyomaviruses, and papillomavirus, have evolved to encode these molecules [19–23], have developed mechanisms to counter host miRNAs, or use host miRNAs to their own advantage [24]. In plants, *Drosophila*, and *C. elegans* Dicer-initiated viral immunity plays a major role in the restriction of viral replication, and viruses have evolved the ability to counteract this defense mechanism [25–27]. In mammals, Dicer knockdown or knockout results in increased susceptibility to certain viruses, like HIV and vesicular stomatitis virus (VSV) due to the suppression of certain host miRNAs that have antiviral activity [24, 28].

The original aim of our study was to explore the role of miRNAs in VACV infection, their target genes and their biologic effects. To our surprise, we found that Dicer1 was completely suppressed in VACV-infected cells, and this was associated with a universal reduction of host miRNAs expression. These observations have prompted this report.

Materials and methods

Cell cultures and RNA extraction

HeLa cells were grown in DMEM medium supplemented with 10 % fetal calf serum (FCS), 1 % L-glutamine and 1 % penicillin/streptomycin (all reagents were supplied by Biological Industries, Beit Haemmek, Israel). Viral stocks of VACV (strain WR) were prepared using HeLa cells, which were grown in the presence of 2 % FCS. The multiplicity of infection (MOI) for the experiments described was 0.5. HeLa cells were mock infected or infected with VACV stock and then harvested at various times postinfection. Total RNA was isolated from cells using an EZ-RNA II kit (Biological Industries, Beit Haemmek, Israel) according to the manufacturer's instructions.

miRNA microarray analysis

To determine the effect of VACV on host miRNA expression, total RNA was extracted from VACV-infected and uninfected HeLa cells. cRNA labeled with either cyanine 3-CTP (Cy3-CTP) or cyanine 5-CTP (Cy5-CTP) was generated from each cDNA source using a Low-Input Linear Amplification Kit (Agilent Technologies, Santa Clara, USA) according to the manufacturer's protocol, except that synthesis was initiated at the *in vitro* transcription step using 1 μg of cDNA as starting material. Hybridization to a chip (MIRCHIP^{TM}, custom made by Agilent Technologies, Santa Clara CA, USA) displaying 45-mer oligonucleotide probes complementary to all human miRNAs, which were printed in triplicate spots, was carried out in solutions that contained the indicated amount of each labeled cRNA from either control or test samples that were prepared using an *in situ* Hybridization Reagent Kit (Agilent). Hybridized microarrays were scanned using an Agilent LP2 DNA Microarray Scanner at 10-μm resolution. Microarray images were visually inspected for defects. To each sample, external spotted controls were added for normalization between samples. Data analysis was carried out by Rosetta Genomics.

Deep sequencing

Deep sequencing was carried out at the Tel Aviv University Genome High-Throughput Sequencing Laboratory following Illumina's Small RNA Sample Preparation Protocol v1.5. The analysis of differential expression was done using miRNAkey software [29].

Detection of host miRNAs by quantitative real-time PCR (qPCR)

Preparation of cDNA libraries and quantitative real-time PCR analysis of miRNAs was done using a miScript reverse transcription kit followed by miScript SYBR Green PCR kit (QIAGEN^{GmbH}, Hilden, Germany). The qPCR Cq values of U6 were used for normalization between samples. Each experiment was repeated three times in duplicate. Statistical analysis was carried out using one-way ANOVA.

Analysis of host gene expression

Following transfection or VACV infection, total RNA was extracted using an EZ-RNA II kit (Biological Industries, Beit Haemmek, Israel, see above) and analyzed for transcripts of vaccinia virus VACV-D9, VACV-D10, host

Table 1 Primers used

Primer	Sequence	Remarks
GFP-VACV-D9-F	GGC**AAGCTT**ATGGGAATTACAATGGATG	pEGFP-C1-D9 cloning
GFP-VACV-D9-R	GGC**GGATCC**CGTTTACTATTAACTAGCATATTATAAATATAAGA	pEGFP-C1-D9 cloning
GFP-VACV-D10-F	GGC**AAGCTT**ATGAACTTTTACAGATCTAGTATAATTAG	pEGFP-C1-D10 cloning
GFP-VACV-D10-R	GGC**GGATCC**CGATCATCCTCAGTTAATTTTTTTAATGA	pEGFP-C1-D10 cloning
Dicer F[a]	TTAACCAGCTGTGGGGAGAGGGCTG	Dicer qRT-PCR
Dicer R[a]	AGCCAGCGATGCAAAGATGGT	Dicer qRT-PCR
Drosha F[b]	CATGCACCAGATTCTCCTGTA	Drosha qRT-PCR
Drosha R[b]	GTCTCCTGCATAACTCAACTG	Drosha qRT-PCR
D9WR F	AAGAACTTCATTCGCGTTCC	D9 qRT-PCR
D9WR R	ATCGATATTGTTGATAGAACCTT	D9 qRT-PCR
D10WR F	TCCAATTATTTGAACAAACAGGAA	D10 qRT-PCR
D10WR R	TCCCTGGATAAACACTCTGGA	D10 qRT-PCR
D9VVL-F	ATGGGAATTACAATGGATGAGGAA	Long qRT-PCR
D9VVL-R	ATCGATATTGTTGATAGAACCTT	Long qRT-PCR
D10VVL-F	TCCAATTATTTGAACAAACAGGAA	Long qRT-PCR
D10VVL-R	GTCCATTACCTGAATTCGGATCT	Long qRT-PCR
pEGFP-C1 F	CATGGTCCTGCTGGAGTTCGTG	Sequencing
pEGFP-C1 R	ATGTTTCAGGTTCAGGGGGAG	Sequencing
β-actin F	TTGCCGACAGGATGCAGAAG	Reference gene
β-actin R	AAGCATTTGCGGTGGACGAT	Reference gene
GAPDH F	ACCACAGTCCATGCCATCAC	Reference gene
GAPDH R	TCCACCACCCTGTTGCTGTA	Reference gene
Pre-miR-130b F	TACTATAGGCCGCTGGGAAG	
Pre-miR-130b R	GACCTGACCGATGCCCTTTCA	
Pre-miR-193a F	CGAGGATGGGAGCTGAGG	
Pre-miR-193a R	CGGGGGCCGAGAACTGGGA	
hsa-miR-130b	CAGTGCAATGATGAAAGGGCA	
hsa-miR-193a	AACTGGCCTACAAAGTCCCAG	

Bold nucleotides are restriction enzyme sites (HindIII and BamHI)

[a] gi 168693431

[b] gi 155030233

β-actin, GAPDH, Drosha and Dicer1 by one-step reverse transcription real-time PCR (RT-qRT-PCR, SUPER III P. SYBR one-step QRT-PCR, Invitrogen, Carlsbad, CA, USA; see Table 1 for details of the primer pairs used). Protein samples were extracted from cells at various times after infection using RIPA buffer for whole-cell extracts. Proteins (10 μg) were separated by 8 % SDS-PAGE and blotted onto a nitrocellulose membrane. Membranes were then probed with either rabbit polyclonal antibody to Dicer1 (I101-HU, a kind gift of K. Rajewsky, Harvard Medical School, USA) or a monoclonal antibody to β-actin (SC-47778, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and peroxidase-labeled goat anti-rabbit IgG or goat anti-mouse, respectively, as secondary antibodies. An ECL kit (Santa Cruz Biotechnology, CA, USA) was used for detection according to the manufacturer's protocol. Densitometry of the film was performed using an imaging densitometer (Bio-Rad Laboratories, Hercules, CA, USA). mRNA expression of Dicer1 and Drosha was normalized to β-actin mRNA expression, protein expression of Dicer1 was standardized to β-actin, and changes in expression of Dicer1, following transfection with D9 or D10 (see below), was normalized to its expression in pEGFP-C1-transfected cells.

Overexpression of VACV D9 and D10 in HeLa cells

Construction of plasmids: DNA was isolated from VACV-infected HeLa cells using a DNA blood purification kit (QIAGEN GmbH, Hilden, Germany) and used as template for PCR. PCR reactions were performed using forward and reverse primers for D9 and D10 (GFP-VACV-D9-F and -D9-R and GFP-VACV-D10-F and -D10-R, respectively, listed in Table 1) according to standard protocols. The amplicons were isolated and ligated to pGEM-T Easy

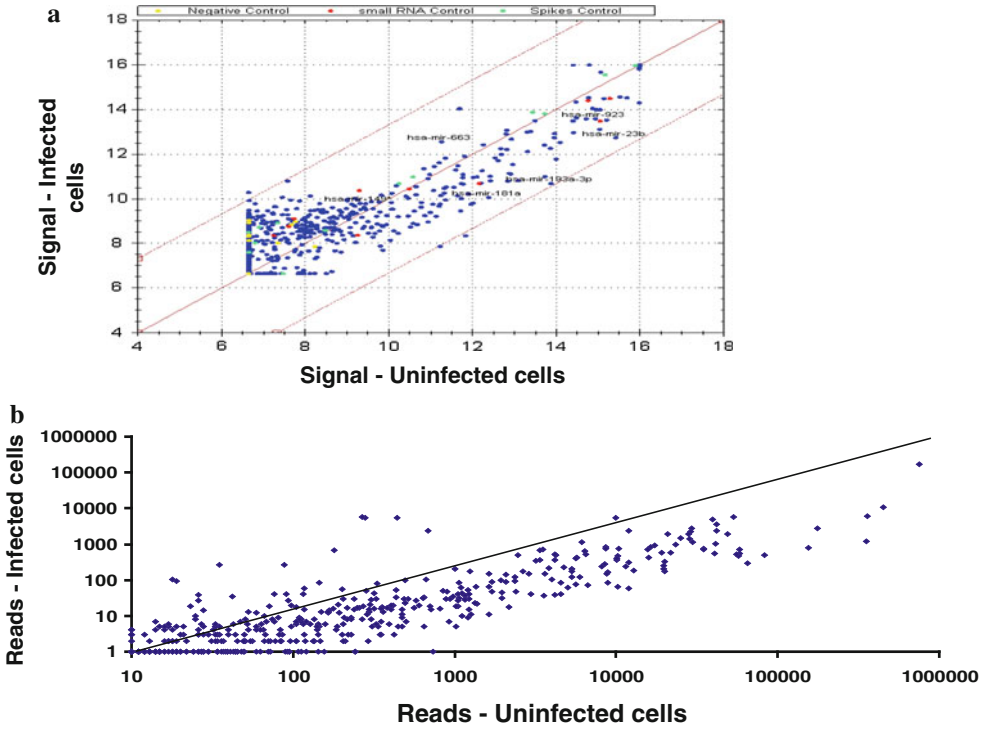

Fig. 1 Decrease of the vast majority of host miRNAs following VACV infection. Microarray (**a**) and deep sequencing (**b**) analysis of human miRNA expression in uninfected vs. VACV-infected HeLa cells were performed (see "Materials and methods" for details). **a** Signals of hsa-miRNAs after 24 h of VACV infection (y-axis) vs. mock-infected cells (x-axis). Shown are the signals obtained using the MIRCHIP after 24 h, from the various times tested (2, 24 and 48 h). **b** Readouts of hsa-miRNAs after 36 h of VACV infection (y-axis) vs. mock-infected cells (x-axis), showing the results one out of two experiments

Vector I (Promega, Madison, USA) according to the manufacturer's instructions. Selected clones were digested with HindIII and BamHI and subcloned into the pEGFP-C1 vector (Clontech Laboratories, Mountain View, USA). Next, HIT competent DH5α cells (RBC Bioscience, Chung Ho City 235, Taipei, Taiwan) were transformed with the ligation mixtures, and kanamycin (Sigma Aldrich, Rehovot, Israel)-resistant colonies were picked and sequenced to ensure the expression of D9 or D10 in the same open reading frame as the GFP protein.

Transfection of HeLa cells with VACV D9 and D10 decapping enzymes: HeLa cells were transfected with either pEGFP-C1-D9 or pEGFP-C1-D10 plasmids, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Briefly, 5×10^6 cells were plated in 10-cm plates for 24 h, and a mixture containing 15 μg DNA and Lipofectamine in OPTI-MEM medium (GIBCO) was added to the cells for 6 hour and then replaced with fresh growth medium. Cells were incubated further for 72 h. Controls cells were transfected with empty pEGFP-C1 vector.

Results

Decreased expression of host miRNAs following vaccinia virus infection

HeLa cells were mock infected or infected with VACV for 2, 24, and 48 h. RNAs were extracted and analyzed to determine the expression of human miRNAs (hsa-miRNAs), using custom made MIRCHIP® (see "Materials and methods" for details) (Figs. 1a, 2a). For each time point, expression of hsa-miRNAs was compared between VACV-infected and mock-infected cells (HeLa). Two hours after infection, there was no difference between the expression of hsa-miRNAs in infected vs. uninfected cells (Fig. 2a). However, after twenty-four hours of VACV infection, a relative decrease of the vast majority of host miRNAs (overall, two- to fourfold) was observed in the infected cells, and a further decrease was observed by 48 h (Figs. 1a, 2a). This phenomenon was general, although, a few miRNAs were either upregulated or showed no differential expression during the infection (e.g., hsa-miR-149*, miR-132, miR-15a). To confirm the results obtained using the MIRCHIP® and to evaluate the results at an additional time point, differential expression of host miRNAs after vaccinia infection was evaluated by deep sequencing (DS) 36 hours postinfection (hpi). The DS readouts of host miRNAs in VACV-infected cells were markedly reduced (Figs. 1b, 2b). The reduced expression of representative differentially expressed miRNAs (Fig. 3) was confirmed by qPCR analysis of VACV-infected vs. uninfected cells ($p = 0.006$) at 48 h after infection. The readouts of hsa-miR130b and hsa-miR193a were reduced from 3556 to 36 reads and from 1111 to 10 (respectively) following VACV infection, and the qPCR analysis showed a two- to four-fold decrease in expression of hsa-miR-193a and hsa-miR-130b (see Fig. 3). This decrease was not due to reduced expression of their precursors, the pre-miRNAs (Fig. 4). On the contrary, the expression of their precursors

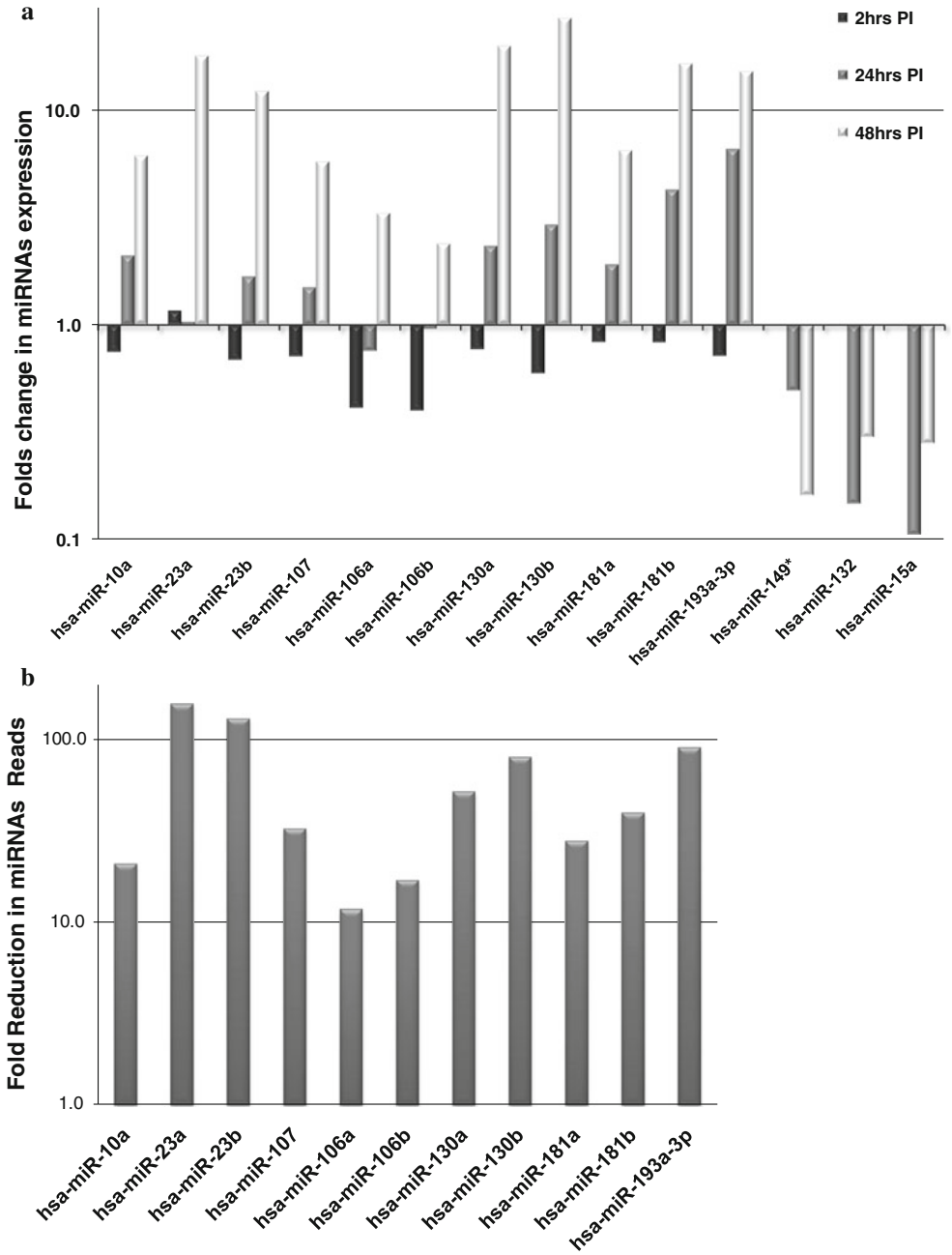

Fig. 2 Gradual decrease of host miRNA expression during VACV infection. Relative expression of miRNAs from infected vs. uninfected host (hsa-mir-10a, 23a, 23b, 107, 106a, 106b, 130a, -130b, 181a, 181b, -193a-3p) at various times after infection. **a** MIRCHIP analysis was done 2 h (*black bars*), 24 h (*gray bars*), and 48 h (*white bars*) postinfection. **b** Deep sequencing analysis was carried out 36 h postinfection (*dark bars*). See "Materials and methods" for details

remained unchanged or even increased by twofold, at 24 h, and 48 h after VACV infection.

Vaccinia virus affects the miRNAs biogenesis pathway

Processing of miRNAs from their precursors, in the nucleus, to their mature form in the cytoplasm is a sequential process in which the Drosha, Exportin 5 and Dicer enzymes play a central role [16]. Although VACV is known to accelerate RNA degradation in infected cells, we measured specifically the expression of enzymes that are involved in miRNAs biogenesis, i.e., Drosha and Dicer1. qPCR and western blot (WB) were used to study the changes in transcription and translation of Dicer1 and transcription of Drosha following VACV infection (Fig. 5). While the expression of mRNA for Drosha (Figs. 5a, 6)

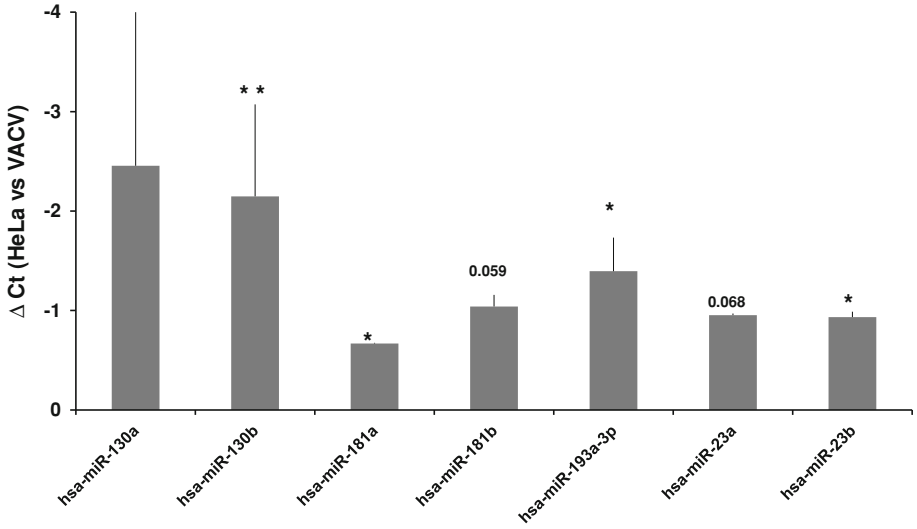

Fig. 3 A relative decrease in host miRNA levels in VACV-infected cells. The relative expression of various hsa-miRNAs in VACV versus mock control HeLa cells was tested by real-time qPCR (see "Materials and methods" for details). The Cq values were normalized to U6 expression and the change in Cq is depicted for seven human miRNAs (hsa-mir-130a, 130b, 181a, 181b, 193a-3p, 23a, 23b) at 24 h post infection. Shown is the average of three experiments done in duplicate (*p ≤ 0.5; **p ≤ 0.01)

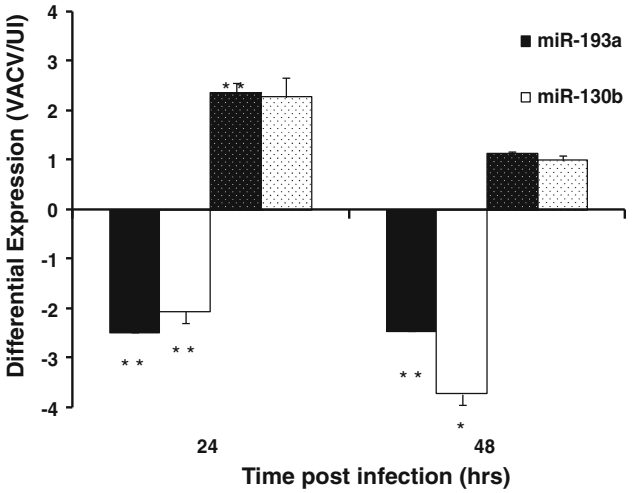

Fig. 4 Host pre-miRNA and miRNA expression in VACV-infected cells. The relative expression of two hsa-miRNAs and their precursors in VACV-infected vs. control HeLa cells was tested by real-time qPCR employing SYBR green (see "Materials and methods" for details). The Cq values were normalized to U6 expression and the changes in Cq (increased or decreased) are depicted for the human miRNA-hsa-130b and its pre-miR (*white bars* and *white bars* with *black dots*, respectively), and for hsa-193a-3p and its pre-miR (*black bars* and *black bars* with *white dots*, respectively), at two different time points postinfection: 24 and 48 h. Shown is the mean ± SD of two experiments (*p ≤ 0.5; **p ≤ 0.01)

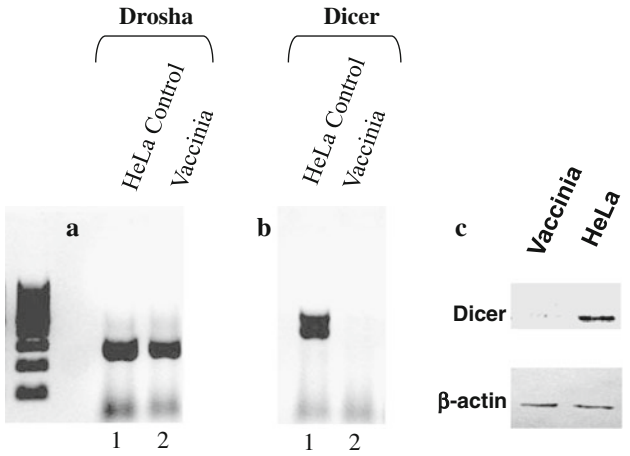

Fig. 5 Abrogation of Dicer1 in VACV-infected cells. SYBR green RT-PCR followed by gel-electrophoresis was performed to measure transcription of Drosha (**a**) and Dicer1 (**b**) mRNAs in VACV-infected (48 h) versus uninfected HeLa cells. Expression of Dicer1 protein in infected and uninfected HeLa cells was detected by western blot analysis using antibodies to probe for Dicer1 and *β*-actin (**c**) expression. Shown are results of one representative experiment out of three

and pre-miRNAs (Fig. 4) remained constant throughout 72 h, the transcript of Dicer1 was abolished within 5 to 10 h after VACV infection (Figs. 5b, 6). The amount of Dicer1 protein decreased gradually, reaching 75 % by 72 h after the infection (Fig. 5c). Suppression of Dicer1 was clearly associated with VACV infection alone and was not dependent on the cell type used, with Dicer being equally suppressed in VACV-infected HeLa, Vero, and 3T3 cells (Supplement Figure 1). Infection of cells with other DNA viruses (herpes simplex type 1 [HSV-1] and type 2 [HSV-2]) or with RNA viruses (influenza A virus and human respiratory syncytial virus [hRSV]) had no effect on Dicer expression (data not shown).

Role of vaccinia virus decapping enzymes

Regulation of host gene expression by VACV is mediated through various mechanisms, one of which is through its decapping enzymes. Since sequential expression of D9 and D10 coincided with the decline in Dicer1 mRNA expression and changes in miRNAs expression, we explored the possibility that these decapping enzymes might affect Dicer1 expression and hsa-miRNAs abrogation. HeLa cells were therefore transiently transfected with plasmids

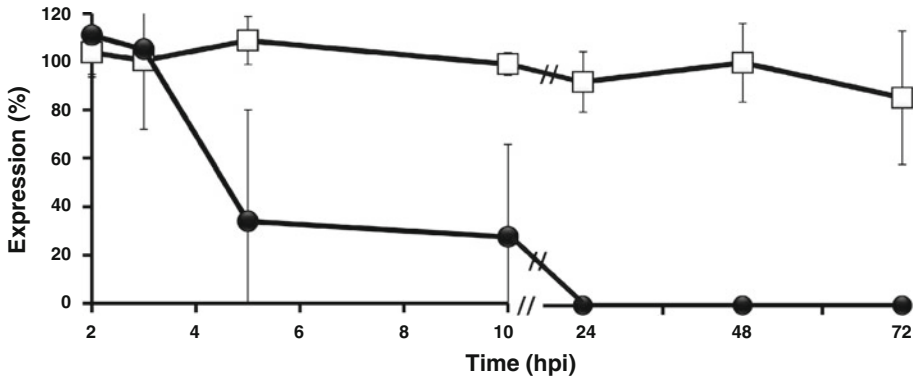

Fig. 6 Time course of Dicer1 expression in VACV-infected cells. SYBR green qRTPCR analysis was performed to determine the relative levels (percentage of those at time zero; y-axis) of Drosha (*white squares*) and Dicer1 (*black circles*) mRNAs in VACV-infected and uninfected HeLa cells at various times after infection (x-axis; 2, 3, 5, 10, 24, 48 and 72 h postinfection [hpi]). Shown is the mean ± SD of four experiments

expressing fusion proteins of either GFP-D9 or GFP-D10 (pEGFP-C1-D9 or pEGFP-C1-D10). Cells transfected with pEGFP-C1 alone or infected with VACV served as controls. Seventy-two hours after transfection, RNAs were extracted from the cells and the transcripts of Dicer1, Drosha, β-actin, and GAPDH were analyzed by qPCR. Overexpression of either D9 or D10 did not significantly reduce mRNA transcription of either Dicer1 or Drosha (Fig. 7a), while VACV infection affected Dicer and miRNAs expression. In addition, abrogated expression of Dicer1 was also observed when cells were infected with VACV D9 and D10 deletion mutants (generously given to us by Dr. B. Moss) (Fig. 7b). Taken together, our results show that the VACV decapping enzymes cannot account for the decrease of host miRNAs through Dicer1 abrogation.

Discussion

We have demonstrated that VACV infection is accompanied by suppression of Dicer1 expression and downregulation of host microRNA biogenesis in a manner that is independent of VACV decapping enzymes. This study therefore highlights a viral mechanism that interferes with the host microRNA machinery. Plants and invertebrates have adopted Dicer-initiated viral immunity as a major part of their defense makeup; concomitantly, their viruses have developed various strategies to cope with Dicer, RISC assembly, and dsRNA recognition by the host [25]. In mammals, adenovirus regulates key enzymes of microRNA biogenesis via VA-RNAs that function as competitive substrates squelching Exportin 5 and Dicer1 [30, 31]. However, siRNA formation by the host against mammalian viruses has not being reported. Previous studies have shown that knockdown or deletion of Dicer1 can promote HIV-1 [24] and VSV [28] infection *in vitro*. These studies attributed viral suppression to one or two distinct host miRNAs that targeted either viral-essential host gene or viral genes. HIV-1 actively suppressed the expression of the polycistronic miRNA cluster miR-17/92. This suppression was found to be required for efficient viral replication and was dependent on the histone acetyltransferase Tat cofactor PCAF which is a target for host miR-17-5p and miR-20a [24]. VSV, on the other hand, was shown to be inhibited by m-miR-24 and -93, which target virus-encoded genes: the large and phosphor proteins (L, P) [28, 32]. Otsuka et al. developed murine *Dicer1*$^{d/d}$-induced knockout in macrophages. Using this model, they were able to determine the effect of Dicer1 and miRNAs biogenesis on the replication of various viruses. Among the viruses tested were encephalomyocarditis virus, lymphocytic choriomeningitis virus, coxsackievirus (group B serotype 3), influenza A virus, herpes simplex type 1 (HSV1), VSV, and VACV. Only VSV and HSV1 viruses showed increased replication in the absence of Dicer1, while VACV replication was not affected by the absence of Dicer1 [24, 28]. The results of our studies can clearly provide an explanation for this observation, since Dicer activity is apparently knocked out by the virus, and Dicer1 knockout in the cells therefore could not improve further viral replication.

While D9 is transcribed early during infection, D10 is an intermediate-late protein [5]. In our study, we could not show that overexpression of either one of them alone or both together resulted in a significant decrease in Dicer1 expression or a significant decrease of host microRNAs. Furthermore, infection of cells with either D9 or D10 deletion mutants of VACV was still associated with decreased Dicer1 expression, to the same extent as with wt virus infection.

Currently, it is not known whether the VACV genome encodes miRNAs or siRNAs, or if host miRNAs promote

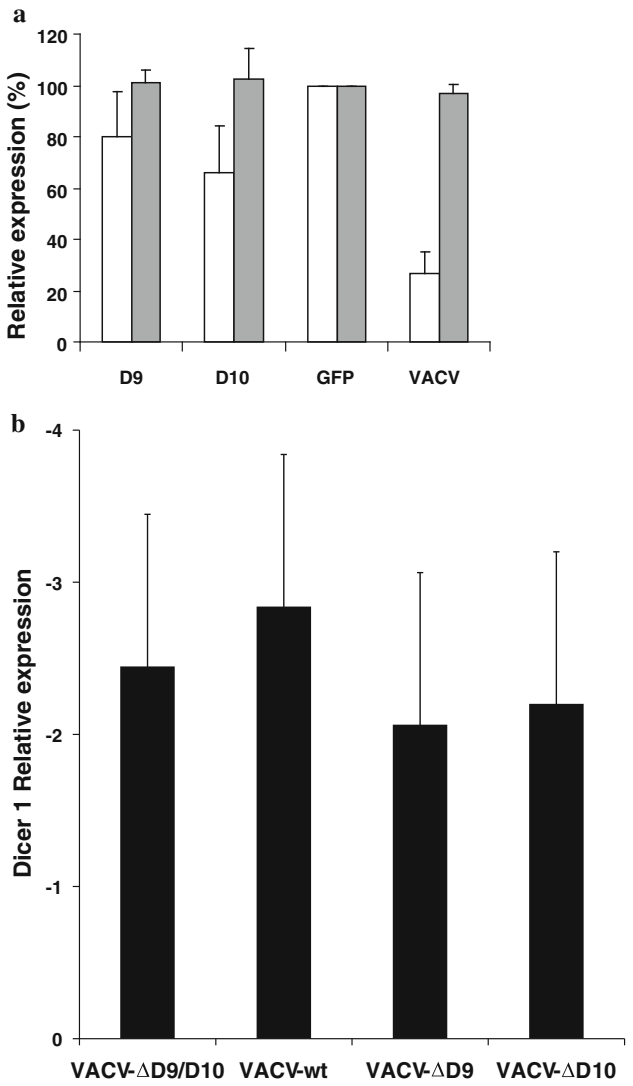

Fig. 7 Dicer1 expression is not affected by D9 or D10. **a** Overexpression of D9 and D10. HeLa cells were transfected with either pEGFP-C1-D9 or pEGFP-C1-D10, for 72 h. Total RNA was extracted (pEGFP-C1-transfected cells and VACV-infected cells served as negative and positive controls). SYBR green qPCR followed by gel electrophoresis was performed to measure mRNA transcription of Dicer1 (*white bars*), and Drosha (*gray bars*). Dicer1 expression in VACV-infected cells (Vac) served as positive control. Shown is the mean ± SD of three experiments. **b** The effect of VACV deletion mutants (D9 and D10) on Dicer1 expression. HeLa cells were either left uninfected or infected with wt-vaccinia, ΔD9 or ΔD10 deletion mutants or both together. Forty-eight h after infection, total RNAs were extracted, and Dicer1 mRNA expression was tested by qRTPCR and compared to the uninfected control. Shown is the mean ± SD of three experiments done in duplicate

or inhibit its replication. VACV infection does not eliminate all miRNAs from infected cells, and recent studies have used miRNA-based gene regulation to suppress B5R expression of oncolytic VACV through let-7a miRNA [33]. However, our data clearly indicate that VACV infection is accompanied by a general suppression of the host microRNA machinery, which is associated with Dicer1 abrogation but is independent of D9 and D10 decapping enzymes. Thus, the suppression of Dicer1 and microRNA expression by VACV may be of central importance in facilitating the infection and overcoming host resistance, although the mechanism responsible for such suppression is not clear and remains to be defined.

Acknowledgment This work was supported by the Israel Science Foundation (grant no. 1375/05) and the Israel Ministry for Science, Culture, and Sport (grant no. 3-4435). The sponsor had no role in the study.

References

1. Moss B (1996) Poxviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM (eds) Fields virology, vol 3. Lippincott-Raven, New York, p 2637
2. Katsafanas GC, Moss B (2007) Colocalization of transcription and translation within cytoplasmic poxvirus factories coordinates viral expression and subjugates host functions. Cell Host Microbe. doi:10.1016/j.chom.2007.08.005
3. Schramm B, Locker JK (2005) Cytoplasmic organization of POXvirus DNA replication. Traffic. doi:10.1111/j.1600-0854.2005.00324.x
4. Parrish S, Resch W, Moss B (2007) Vaccinia virus D10 protein has mRNA decapping activity, providing a mechanism for control of host and viral gene expression. Proc Natl Acad Sci USA. doi:10.1073/pnas.0611685104
5. Parrish S, Moss B (2007) Characterization of a second vaccinia virus mRNA-decapping enzyme conserved in poxviruses. J Virol. doi:10.1128/JVI.01668-07
6. Boone RF, Parr RP, Moss B (1979) Intermolecular duplexes formed from polyadenylylated vaccinia virus RNA. J Virol 30:365–374
7. Arsenio J, Deschambault Y, Cao J (2008) Antagonizing activity of vaccinia virus E3L against human interferons in Huh7 cells. Virology. doi:10.1016/j.virol.2008.04.014
8. Carroll K, Elroy-Stein O, Moss B, Jagus R (1993) Recombinant vaccinia virus K3L gene product prevents activation of double-stranded RNA-dependent, initiation factor 2 alpha-specific protein kinase. J Biol Chem 268:12837–12842
9. Chang HW, Watson JC, Jacobs BL (1992) The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proc Natl Acad Sci USA 89:4825–4829
10. Haga IR, Bowie AG (2005) Evasion of innate immunity by vaccinia virus. Parasitology. doi:10.1017/S0031182005008127
11. Rivas C, Gil J, Melkova Z, Esteban M, Diaz-Guerra M (1998) Vaccinia virus E3L protein is an inhibitor of the interferon (i.f.n.)-induced 2–5A synthetase enzyme. Virology 248:406–414
12. Wang J, Xu R, Lin F, Zhang S, Zhang G, Hu S, Zheng Z (2008) MicroRNA: Novel Regulators Involved in the Remodeling and Reverse Remodeling of the Heart. Cardiology. doi:10.1159/000172616
13. Schickel R, Boyerinas B, Park SM, Peter ME (2008) MicroRNAs: key players in the immune system, differentiation, tumorigenesis and cell death. Oncogene. doi:10.1038/onc.2008.274
14. Chang J, Guo JT, Jiang D, Guo H, Taylor JM, Block TM (2008) Liver-specific microRNA miR-122 enhances the replication of hepatitis C virus in nonhepatic cells. J Virol. doi:10.1128/JVI.02575-07

15. Kim VN (2005) MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol. doi:10.1038/nrm1644
16. Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. Cell. doi:10.1016/j.cell.2009.01.035
17. Hammond SM, Bernstein E, Beach D, Hannon GJ (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. doi:10.1038/35005107
18. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297
19. Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR (2005) Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci USA. doi:10.1073/pnas.0408192102
20. Cai X, Schafer A, Lu S, Bilello JP, Desrosiers RC, Edwards R, Raab-Traub N, Cullen BR (2006) Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. PLoS Pathog. doi:10.1371/journal.ppat.0020023
21. Grey F, Nelson J (2008) Identification and function of human cytomegalovirus microRNAs. J Clin Virol. doi:10.1016/j.jcv.2007.11.024
22. Pfeffer S, Zavolan M, Grasser FA, Chien M, Russo JJ, Ju J, John B, Enright AJ, Marks D, Sander C, Tuschl T (2004) Identification of virus-encoded microRNAs. Science. doi:10.1126/science.1096781
23. Umbach JL, Nagel MA, Cohrs RJ, Gilden DH, Cullen BR (2009) Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. J Virol. doi:10.1128/JVI.01185-09
24. Triboulet R, Mari B, Lin YL, Chable-Bessia C, Bennasser Y, Lebrigand K, Cardinaud B, Maurin T, Barbry P, Baillat V, Reynes J, Corbeau P, Jeang KT, Benkirane M (2007) Suppression of microRNA-silencing pathway by HIV-1 during virus replication. Science. doi:10.1126/science.1136319
25. Aliyari R, Ding SW (2009) RNA-based viral immunity initiated by the Dicer family of host immune receptors. Immunol Rev. doi:10.1111/j.1600-065X.2008.00722.x
26. Obbard DJ, Gordon KH, Buck AH, Jiggins FM (2009) The evolution of RNAi as a defence against viruses and transposable elements. Philos Trans R Soc Lond B Biol Sci. doi:10.1098/rstb.2008.0168
27. van Rij RP, Berezikov E (2009) Small RNAs and the control of transposons and viruses in Drosophila. Trends Microbiol. doi:10.1016/j.tim.2009.01.003
28. Otsuka M, Jing Q, Georgel P, New L, Chen J, Mols J, Kang YJ, Jiang Z, Du X, Cook R, Das SC, Pattnaik AK, Beutler B, Han J (2007) Hypersusceptibility to vesicular stomatitis virus infection in Dicer1-deficient mice is due to impaired miR24 and miR93 expression. Immunity. doi:10.1016/j.immuni.2007.05.014
29. Ronen R, Gan I, Modai S, Sukacheov A, Dror G, Halperin E, Shomron N (2010) miRNAkey: a software for microRNA deep sequencing analysis. Bioinformatics. doi:10.1093/bioinformatics/btq493
30. Lu S, Cullen BR (2004) Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. J Virol. doi:10.1128/JVI.78.23.12868-12876.2004
31. Andersson MG, Haasnoot PC, Xu N, Berenjian S, Berkhout B, Akusjarvi G (2005) Suppression of RNA interference by adenovirus virus-associated RNA. J Virol. doi:10.1128/JVI.79.15.9556-9565.2005
32. Muller S, Imler JL (2007) Dicing with viruses: microRNAs as antiviral factors. Immunity. doi:10.1016/j.immuni.2007.07.003
33. Hikichi M, Kidokoro M, Haraguchi T, Iba H, Shida H, Tahara H, Nakamura T (2011) MicroRNA regulation of glycoprotein B5R in oncolytic vaccinia virus reduces viral pathogenicity without impairing its antitumor efficacy. Mol Ther. doi:10.1038/mt.2011.36