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Acyclovir and A β 42 peptide attenuates HSV-1-induced miRNA-146a levels in human brain cells

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Abstract

Human brains harbor herpes simplex virus type-1 (HSV-1) DNA which normally remains quiescent throughout many decades of life. HSV-1 is associated with viral encephalopathy and with the amyloid beta 42 (A β 42) peptide-enriched lesions that characterize Alzheimer's disease neuropathology. Here we report that infection of human neuronal-glial (HNG) cells in primary co-culture with HSV-1 induces an irregular hypertrophy of HNG cell bodies, an induction of HSV-1 DNA polymerase, and an up-regulation of micro-RNA-146a associated with altered innate-immune responses. Presence of the antiviral acyclovir or soluble A β 42 peptide significantly attenuated these neuropathological responses. The inhibitory effects of A β 42 peptide were also observed in an HSV-1-infected CV-1 cell-based viral plaque assay. The results suggest that soluble A β 42 peptide can invoke non-pathological and anti-viral effects via inactivation of an HSV-1 challenge to human brain cells by simple viral sequestration or by complex neurogenetic mechanisms.

Keywords

acyclovir (ACV); acylguanosine; Alzheimer's disease; amyloid beta 42 peptide (A β 42); herpes simplex virus type-1 (HSV-1); innate immune response; inflammation; miRNA-146a

INTRODUCTION

Herpes simplex virus-1 (HSV-1), a neuro-invasive Group 1 member of the herpes virus family *Herpesviridae*, is known to establish lifelong latency in human nervous tissues [1,2]. HSV-1 neural activation is associated with viral encephalopathy, the induction of altered innate immune and inflammatory responses, immunosuppression and neurodegeneration [1–5]. Although a link between HSV-1 infectivity, latency and Alzheimer's disease has long

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been suspected, and HSV-1 associates with amyloid-beta 42 (A β 42) peptides that characterize Alzheimer's disease neuropathology, the molecular-genetic mechanism of the HSV-1-A β 42 peptide association is not fully understood [3–7].

Micro-RNAs (miRNAs) are small, non-coding RNAs that through base-pair complementarity and hydrogen bonding with their target messenger RNAs (mRNAs) regulate miRNA-mRNA expression networks in health and disease [8–12]. Brain cells utilize only a specific subset of all known human miRNAs in normal homeostatic neural functions [8,9], and mis-regulation of several brain-enriched miRNAs appear to contribute to neurodegeneration and neurological disease [3,9–14]. Specific miRNAs such as miRNA146a have been found to be significantly up-regulated during HSV-1 and other viral infections to function in the regulation of oxidative stress, host inflammatory signaling and innate immunity [8–14].

In this study we used human miRNA arrays, Northern dot blot, RT-PCR for HSV-1 DNA polymerase, and viral infectivity assay to examine the effects of the 'classical' antiviral acycloguanosine acyclovir (ACV) and soluble A β 42 peptide on HSV-1 (17syn+) infection and miRNA-146a regulation in 2.5 week old human neuronal-glia (HNG) cells in primary co-culture. The results suggest that both ACV and A β 42 peptide effectively quench neuroinvasive- and neuroinfective-associated actions of HSV-1 and their ensuing pathogenic effects, in part by blocking the HSV-1-mediated induction of miRNA-146a. The results further suggest that soluble A β 42 peptides are capable of invoking non-pathological and anti-viral effects in sequestering or inactivating an HSV-1 challenge to primary human brain cells, either by simple A β 42 peptide-HSV-1 adsorption and sequestration, or via other complex, yet unidentified, neurogenetic mechanisms.

Materials and Methods

Reagents and synthetic oligonucleotides and peptides

All reagents used were obtained from commercial suppliers and were used without further purification. Synthetic 22 nucleotide sequences encoding miRNA-132 and miRNA-146a were obtained from Applied Biosystems/Ambion (Austin, TX) or Invitrogen (Carlsbad, CA). A β 42 peptide and a biologically inactive A β 42 scrambled (A β 42s) control peptide were obtained from American Peptide (Sunnyvale, CA) and used at 5 and 100 μ M concentrations in cell culture media; acycloguanosine, as acyclovir (ACV) was obtained from Sigma-Aldrich (St. Louis, MO) and used at 30 μ M. RNase-free plasticware and RNase-free isolation reagents including diethyl pyrocarbonate (DEPC) water were purchased from Ambion, Invitrogen or Sigma-Aldrich and were used as previously described [3,10].

Human neuronal-glia (HNG) cells in primary culture, CV-1 cells and HSV-1 infection

HNG cells (CC-2599; Lonza Corporation, Walkersville, MD) were cultured in an HNG maintenance medium (HNMM) for 2.5 weeks as previously described by our group [3,10–12] (Fig. 1A). HNG co-cultures are a useful bio-platform to study basic disease mechanisms and drug efficacy as human primary neuronal cultures do not culture well in the absence of glia [3,9–12; unpublished observations]. At 2.5 weeks HNG cells displayed approximately equal populations of neurons and glia [10]. CV-1 cells, derived from a simian kidney epithelial cell line and used widely as a host for HSV-1 proliferation studies were obtained from the American Type Culture Collection and cultured according to the supplier's instructions (ATCC; CCL-70; ATCC, Manassas, VA). A low passage 3X plaque purified HSV-1 strain 17 syn+ [15], was added at time '0' at a multiplicity of infection (MOI) ratios of 5:1 or 10:1 (HSV-1 virus particle-to-HN cell ratio) as previously described [3]. HNG cell

morphology was examined using phase contrast microscopy at 0, 24, 48 and 72 hrs post HSV-1 infection using a Nikon Diaphot 200 microscope (Nikon).

CV-1 cell-based antiviral assay

HSV-1 strain 17Syn⁺ was separately pre-incubated with differing concentrations (5 or 100 μ M) of A β 42 or A β 42s (scrambled) peptide in ~200 μ l at 37°C for 1 hr [3,15]. Following peptide pre-treatment, 1x DMEM medium (Cellgro, Mediatech Inc, Manassas, VA) containing 10% FBS and anti-HSV-1 human IgG was added to the ~200 μ l volumes to a final volume of 2 ml. Each was added to an individual well of 6 well-plate of near confluent (80%) monolayer CV-1 cells. An additional control was pre-incubated in which A β 42 or A β 42s peptide was replaced with 1X DMEM; these were plated in triplicate and incubated for 72 hr at 37°C and 5% CO₂. CV-1 cells were fixed with 10% formaldehyde, washed, stained with crystal violet (Hucker's modified solution diluted 1:10 with 20% ethanol and 80% H₂O) and plaques were counted [3,15].

RNA extraction, quality control, and Northern analysis

Total RNA was extracted at 0 time and at 48 hrs after HSV-1 infection as these time points were previously shown to exhibit significant differences both at the morphological and molecular level [3]. At 0 time and 48 hrs post HSV-1 treatment HNG cells were rapidly transferred into 1 ml ice-cold TRIzol reagent (Invitrogen) and total RNA was extracted as previously described [3,10]. RNA quality was determined using an Agilent Bioanalyzer 2100 (Lucent Technologies and Caliper Technologies, Palo Alto, CA) and electropherograms were generated for each total RNA sample [3,9–11]. As a quality control index, if 28S/18S ratios were larger than 1.4 and A_{260/280} $\geq$ 2.1 (indicating high total RNA spectral quality), the samples were used for Northern dot blot analysis [3,9–12]. Samples were analyzed individually or as pooled samples. There were no significant differences in the total RNA yield or RNA spectral quality between the control and HSV-1 infected HNG cells.

Real-Time Quantitative PCR for HSV-1 DNA polymerase

HSV-1 DNA polymerase was analyzed in total RNA samples using a One-Step RT-PCR kit (Invitrogen, Carlsbad, CA) using the forward and reverse primers 5'-CATCACCGACCCGAGAGGGAC-3' and 5'-GGGCCAGGCGCTTGTTGGTGTA-3' [2,16]. The fluorescent probe sequence 5'-6FAM-CCGCCGAAGTGAAGCAGACACCCGCGC-BHQ-1-3' was further used to quantify HSV-1 DNA polymerase signal levels as previously described [2,16].

HNG cell-enriched miRNA array and Northern analysis

Micro-RNA arrays (LC Sciences, Houston, TX) were probed with total small miRNAs isolated at 0 and 48 hrs from control or HSV-1 infected HNG cells. Specific miRNAs showing strong hybridization signals were studied further and subjected to Northern dot blot analysis as previously described [3,10].

Statistical analysis and data interpretation

All statistical procedures for micro RNA and Northern abundance were analyzed using a two-way factorial analysis of variance (*p*, ANOVA) using programs and procedures in the SAS language (Statistical Analysis Institute, Cary, NC) and as previously described by our group [10]. Only *p*-values of less than 0.05 (ANOVA) were considered to be statistically significant. Figures were generated using Excel 2008 (Microsoft Corporation, Redmond, WA), Adobe Illustrator CS3 ver 11.0 and Photoshop CS2 ver 9.0.2 (Adobe, San Jose, CA).

RESULTS

Changes in HNG cell morphology accompany HSV-1 infection; effects of ACV, A β 42 and A β 42s peptides

Control HNG cells (uninfected, untreated) in primary culture exhibited a progressive increase in cell body number and neurite extensions, and after 2.5 weeks of culture exhibited about equal populations of neurons and glia (Fig 1A,B) [11,12]. In contrast, dramatic changes in HNG cell culture morphology were observed 48 hrs post HSV-1 infection typically showing a heterogeneous ‘blebbing’ and hypotrophy as HSV-1 infection progressed from 0 to 48 hr (Fig.1C,D). HNG cells infected with HSV-1 for 48 hrs exhibited extremely distorted cell morphology to the extent that many HNG cell bodies disappeared (data not shown). HNG cells treated with HSV-1 and ACV (30 μ M) or A β 42 peptide (100 μ M) exhibited protection from morphological change (Fig. 1E,F; Fig 1G,H, respectively). Importantly, HNG cells treated with the non-bioactive scrambled A β 42s elicited no protection from the effects of HSV-1 infection (Fig. 1I,J).

Detection of HSV-1 DNA polymerase using RT-PCR

To further ascertain the antiviral actions of A β 42 peptide we assayed for HSV-1 DNA polymerase abundance in HNG cells and the results are shown in Table 1. Consistent with our findings on changes in HNG cell morphology (Fig. 1), 0 copy number of HSV-1 DNA polymerase were found in control, ACV-treated or A β 42-treated HNG cells; there was no significant difference in the detectable abundance of HSV-1 DNA polymerase in HSV-1-infected HNG cells either in the presence or absence of A β 42s.

Antiviral activity of A β 42 peptide using CV-1 cell viral plaque assay

We further studied the inhibitory actions of A β 42 peptide of HSV-1 infection using a CV-1 cell-based viral plaque assay. Control (no peptide) gave an average number of plaques in CV-1 culture of 34.5; the presence of A β 42 peptide at 5 and 100 μ M ambient concentration reduced the average number of plaques to 47% and 16% of control respectively (Table 2). The presence of A β 42s at 5 μ M and 100 μ M ambient concentration showed no anti-viral activity and the average number of plaque counts were not significantly different from controls (Table 2).

RNA quality control and changes in micro-RNA abundance

Both controls and HSV-1-treated HNG cells yielded total RNA samples with 28S/18S ratios larger than 1.4 and $A_{260/280} \geq 2.1$ indicating high spectral quality RNA. Analysis of miRNA panels displaying 911 control RNAs, small RNAs, and miRNA levels and Northern dot blot analysis (Fig 2A-C) indicated that levels of miRNA-146a were consistently up-regulated 4.5-fold, 48 hrs after HSV-1 infection. No changes were observed in the abundance of a brain abundant miRNA-134. All miRNA levels were normalized against 5S RNA in each sample and against 8 hybridization controls on the miRNA panels whose expression levels remained unchanged either before or after HSV-1 infection (Fig 2D) [3]. In agreement with the results obtained using phase contrast microscopy and morphological observations (Fig. 1), miRNA array assay, Northern dot blot assay, HSV-1 DNA polymerase assay (Table 1) and CV-1 cell viral plaque assay (Table 2) the presence of ACV or A β 42 peptide quenched the induction of miRNA-146a but had no effect on miRNA-134 and other miRNAs (data not shown) (Fig. 2E). Importantly, the presence of a non-biologically active peptide control, A β 42s, had no significant effect on miRNA-146a being induced by HSV-1.

DISCUSSION

HSV-1 infection of human neuronal-glia (HNG) co-cultures

Human HNG cells are highly susceptible to infection via neuroinvasive viruses such as HSV-1, a 152 kilobase, linear, double-stranded DNA encoding ~74 genes encased in a 100 nm diameter icosahedral capsid [1,3,17]. Capsid glycoproteins interact with HNG host cell receptors to mediate viral entry and infectivity. Various strains of HSV-1, commonly detected in human nervous tissue, can be stratified into low- and high-reactivation phenotypes; high phenotypic re-activators include HSV-1 strains 17syn+ and McKrae and exhibit high reactivation frequency when induced by physiological stress [1,3,15,16]. As expected, an infection of HSV-1 (17syn+) in HNG cells produced a typical progressive loss of processes and cell rounding or hypertrophy (Fig.1) that have been previously characterized [1–3]. **Effects of A β 42 on expression of HSV-1 DNA polymerase:** HSV-1 DNA polymerase encodes a DNA copying enzyme essential for replication of HSV-1, therefore detection of HSV-1 DNA polymerase can be used as a marker for the success of HSV-1 infectivity [15–17]. This activity was significantly quenched in the presence of ACV and A β 42 peptide. Interestingly, A β 42 peptides, as central mediators of Alzheimer's disease neuropathology, tend to adsorb a wide variety of intracellular and extracellular ligands as they aggregate in Alzheimer A β 42 peptide-enriched senile plaque formations. These results further suggest that a natural increase in A β 42 peptide abundance to a perceived viral infection could be mediated by the brain's innate immune system leading to a pathogenic inflammatory response.

HSV-1 infection of CV-1 cells

CV-1 cells, epithelial cells widely used as a host for HSV-1 proliferation and viral quantitation assay were utilized to study the effects of ACV and A β 42 peptide in a non-neural cell type. That HSV-1-infected cells treated with ACV or A β 42 peptides were again similar to uninfected control HNG cells underscores the potent anti-viral plaque-inducing properties of both ACV and A β 42 peptides.

Regulation of miRNA-146a in HSV-1-infected HNG cells

Micro RNAs are regulatory RNAs acting at the post-transcriptional level, that play critical roles in physiological and patho-physiological processes. An inducible, mouse and human brain abundant miRNA-146a has been implicated as a negative regulator of the innate immune, inflammatory and anti-viral pathway responses in several human diseases including Alzheimer's and prion disease [8–12,18]. Specifically, miRNA-146a up-regulation is associated with down-regulation of complement factor H, an important repressor of the complement signaling cascade [3,9–12]. Interestingly, Kaposi's sarcoma-associated herpesvirus, Epstein-Barr virus and vesicular stomatitis virus have each been shown to up-regulate host miRNA-146a, suggesting a broad role for miRNA-146a in the regulation of antiviral immunity [13,18–21]. In addition to host miRNAs, HSV-1 has been reported to induce specific viral miRNAs, the functions of which are not completely understood [19,22].

Several important questions remain. The mechanism of how A β 42 peptides quench HSV-1-induced changes in HNG cells could operate by multiple interrelated mechanisms. Our results showed maximal effects of soluble A β 42 peptide at 100 μ M ambient concentrations, which is in vast excess to what would be normally present in any stressed HNG or Alzheimer-affected brain cell. Other biophysical forms of A β 42 peptide such as A β 42 fibrils need to be examined for anti-HSV-1 efficacy. By virtue of their amino acid sequence A β 42 peptides are intensely hydrophobic and may simply adsorb HSV-1, and hence sequester HSV-1, to effectively 'immobilize' HSV-1 bioactivity. HSV-1-infected cells produce significant quantities of non-infectious, non-DNA-containing light particles (L-particles)

comprised of viral envelope and tegument proteins, and HSV-1 and L-particles are both integrally associated with A β 42 peptide deposits in Alzheimer brains [6,7]. Alternately, soluble A β 42 peptide could adsorb to HNG outer cell surfaces to inhibit HSV-1 glycoprotein-mediated viral attachment and entry, or there may be a combination of both biophysical events. Recently A β 42 peptides have been shown to evoke potent anti-microbial activities [23]. Although the basis for this bioactivity is not known both antimicrobial and antiviral properties of A β 42 peptides may have common or interrelated bio-protective mechanisms [23,24]. It is conceivable that evolutionary, innate immune or inflammatory adaptations incorporated by human brain cells to successfully evade HSV-1 attack, in part through the up-regulation of A β 42 peptides, could also trigger processes that lead to chronic, neurodegenerative diseases.

CONCLUSION

We demonstrate a protection against induced pathological changes in HSV-1-infected HNG cells using an established antiviral acycloguanosine ACV and soluble A β 42 peptides. While ACV acts as a 'classical' inhibitor of HSV-1 DNA polymerase and hence viral proliferation, the intensely hydrophobic and 'sticky' A β 42 peptide may adsorb and sequester HSV-1, preventing active HNG cell infection. As do several other neuro-invasive viruses, HSV-1 up-regulates miRNA-146a; however, in the presence of either ACV or A β 42 peptide miRNA-146a up-regulation was not observed. Non-pathological effects of A β 42 peptides in the brain and alternative roles for A β 42 peptides in Alzheimer's disease are beginning to be appreciated [23–25]. Anti-viral drug, peptide or anti-miRNA strategies, or their combination, could be useful in blocking the success of HSV-1 infection.

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REFERENCES

1. Whitley, RJ.; Roizman, B. Herpes simplex virus. In: Richman, DD.; Whitley, RJ.; Hayden, FG., editors. Clinical Virology. Washington DC: ASM Press; 2009. p. 409–436.
2. Toma HS, Murina AT, Areaux RG, Neumann DM, Bhattacharjee PS, Foster TP, et al. Ocular HSV-1 latency, reactivation and recurrent disease. *Semin. Ophthalmol.* 2008; 23:249–273. [PubMed: 18584563]
3. Hill JM, Zhao Y, Clement C, Neumann DM, Lukiw WJ. HSV-1 infection of human brain cells induces miRNA-146a and Alzheimer-type inflammatory signaling. *Neuroreport.* 2009; 20:1500–1505. [PubMed: 19801956]
4. Ball MJ. Limbic predilection in Alzheimer dementia: is reactivated herpesvirus involved? *Can. J. Neurol. Sci.* 1982; 9:303–306. [PubMed: 7116237]
5. Wozniak MA, Itzhaki RF. Antiviral agents in Alzheimer's disease: hope for the future? *Therapeutic Advances in Neurological Disorders.* 2010; 3:141–152. [PubMed: 21179606]
6. Kammerman EM, Neumann DM, Ball MJ, Lukiw W, Hill JM. Senile plaques in Alzheimer's disease brains: possible association of beta-amyloid with herpes simplex virus type 1 (HSV-1) L-particles. *Med. Hypotheses.* 2006; 66:294–299. [PubMed: 16242250]
7. Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J. Pathol.* 2009; 217:131–138. [PubMed: 18973185]

8. Provost P. Micro-RNAs as a molecular basis for mental retardation, Alzheimer's and prion diseases. *Brain Res.* 2010; 1338:58–66. [PubMed: 20347722]
9. Lukiw WJ. Micro RNA speciation in fetal, aged and Alzheimer hippocampus. *Neuroreport.* 2007; 18:297–300. [PubMed: 17314675]
10. Lukiw WJ, Zhao Y, Cui JG. An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. *J. Biol Chem.* 2008; 283:31315–31322. [PubMed: 18801740]
11. Sethi P, Lukiw WJ. Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. *Neurosci. Lettters.* 2009; 459:100–104.
12. Lukiw WJ, Pogue AI. Induction of specific miRNA species by ROS-generating metal sulfates in primary human brain cells. *J. Inorg. Biochem.* 2007; 101:1265–1269. [PubMed: 17629564]
13. Lagos D, Pollara G, Henderson S, Gratrix F, Fabani M, Milne RS, et al. miRNA-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. *Nat. Cell Biol.* 2010; 12:513–519. [PubMed: 20418869]
14. Sonntag KC. MicroRNAs and deregulated gene expression networks in neurodegeneration. *Brain Res.* 2010; 1338:48–57. [PubMed: 20380815]
15. Bloom DC, Devi-Rao GB, Hill JM, Stevens JG, Wagner EK. Molecular analysis of HSV-1 during epinephrine-induced reactivation of latently infected rabbits in vivo. *J. Virol.* 1994; 68:1283–1292. [PubMed: 8107194]
16. Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW, Hill JM. HSV-1 DNA in tears and saliva of normal adults. *Invest. Ophthalmol. Vis.Sci.* 2005; 46:241–247. [PubMed: 15623779]
17. Boehmer PE, Villani G. Herpes simplex virus type-1: a model for genome transactions. *Prog. Nucleic Acid Res. Mol. Biol.* 2003; 75:139–171. [PubMed: 14604012]
18. Williams AE, Perry MM, Moschos SA, Larner-Svensson HM, Lindsay MA. Role of miRNA-146a in the regulation of the innate immune response. *Biochem. Soc. Trans.* 2008; 36:1211–1215. [PubMed: 19021527]
19. Li L, Chen XP, Li YJ. MicroRNA-146a and human disease. *Scand. J. Immunol.* 2010; 71:227–231. [PubMed: 20384865]
20. Hou J, Wang P, Lin L, Liu X, Ma F, An H, et al. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. *J. Immunol.* 2009; 183:2150–2158. [PubMed: 19596990]
21. Motsch N, Pfuhl T, Mrazek J, Barth S, Grässer FA. Epstein-Barr virus-encoded latent membrane protein 1 (LMP1) induces the expression of the cellular miRNA-146a. *RNA Biology.* 2007; 4:131–137. [PubMed: 18347435]
22. Cullen BR. Viral and cellular messenger RNA targets of viral microRNAs. *Nature.* 2009; 457:421–425. [PubMed: 19158788]
23. Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, et al. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS One.* 2010; 5:e9505. [PubMed: 20209079]
24. Castellani RJ, Lee HG, Siedlak SL, Nunomura A, Hayashi T, Nakamura M, et al. Reexamining Alzheimer's disease: evidence for a protective role for amyloid-beta protein precursor and amyloid-beta. *J. Alzheimer's Dis.* 2009; 18:447–452.
25. Mondragón-Rodríguez S, Basurto-Islas G, Lee HG, Perry G, Zhu X, Castellani RJ, et al. Causes versus effects: the increasing complexities of Alzheimer's disease pathogenesis. *Expert Rev. Neurother.* 2010; 10:683–691. [PubMed: 20420489]

Figure 1. Significant changes in HNG cell morphology after infection with HSV-1

Morphology of 2.5 week old control HN cells (**A,C,E,G,I**), untreated control HNG cells after 48 hr (**B**), HNG cells 48 hr after infection with HSV-1 (**D**), HNG cells after infection with HSV-1 and treatment with acyclovir (ACV; 30 μ M) (**F**), HNG cells after infection with HSV-1 in the presence of soluble A β 42 (100 μ M) (**H**) or after infection with HSV-1 and treatment a scrambled A β 42 peptide (A β 42s; 100 μ M) (**J**). A β 42s exhibits no known biological activity. In conditions (**D**) and (**J**) note hypertrophy, rounding up of neural cell bodies, atrophy and distortion of neurite extensions, cellular blebbing, and loss of normal HN cell morphology (arrows); 20X magnification. Both ACV (**F**) and A β 42 peptide (**H**)

showed significant protection of HNG cells against these HSV-1-induced pathological changes.

Figure 2.

Human miRNA array panels indicate no change in the expression of miRNA-134 and specific up-regulation of miRNA-146a in HSV-1-treated HNG cells (**A**); absence of miRNA-146a up-regulation in ACV-treated (**B**) or Aβ42 peptide treated HNG cells (**C**) (arrows). Individual miRNA panels were probed with total miRNA obtained from control and HSV-1 infected HNG cells at 48 hrs (**A**) and results were compared; by convention, brown-green fluorescence indicates no difference in expression detected and red fluorescence indicates signal up-regulation. Identity of all miRNAs and their coordinates (**Fig 2 A-C**; rows **a-e**) can be found at www.lsciences.com [3,10]. Independent confirmation of 5S RNA, miRNA-134 and miRNA-146a levels using Northern dot blot

analysis as previously described [3,10] (**D**). No change in the abundance of a control miRNA-134 in any of the samples analyzed was noted. All miRNA levels were normalized against the abundance of 5S RNA in each sample, and also against 8 hybridization controls on the miRNA panels whose expression levels remained unchanged either before or after HSV-1 infection [3]. Signal quantitation for miRNA Northern dot blots from (**D**) compared to 5S RNA in the same sample are shown in (**E**). On the miRNA dot blot panels in (**D**) 5S RNA was loaded at one twentieth the concentration of miRNA-134 and miRNA-146a; N=4; significance over HSV-1-untreated controls; * $p<0.01$ (ANOVA).

Table 1

Detection of HSV-1 DNA polymerase using RT-PCR

average	
condition	copy number [*]
control (no HSV-1)	0
+ HSV-1	1700
+ HSV-1 +ACV	0
+ HSV-1 +Aβ42	0
+ HSV-1 +Aβ42s	1900

* calculated copy number of HSV-1 DNA polymerase per well of HNG cells 48 hr post HSV-1 infection

Table 2Antiviral activity of A β 42 peptide using CV-1 cell viral assay

antiviral peptide		average number of plaques
type of peptide	concentration (μ M)	
no peptide (control)	0	34.5
A β 42s **	5	37.5
A β 42s	100	38.5
A β 42	5	16.5
A β 42	100	5.5

** A β 42s is a scrambled A β 42 peptide with no known biological activity; all HNG cell wells contained HSV-1 plus or minus A β 42 or A β 42s peptides