Oncolytic HSV-1 mediated regulation of the host hypoxia response

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Abstract

Glioblastoma (GBM) is the most common and deadly primary brain tumor, accounting for over 70% of new cancer diagnoses in the United States each year. The poor prognosis for GBM patients necessitates novel biological treatments. One approach examined is the use of oncolytic herpes simplex virus 1 (oHSV). Like many novel treatments, oHSV therapy causes side effects that are not yet well understood. Our lab has demonstrated that oHSV treatment increases the viral ability to downregulate FIH and induces the NF-kB pathway. Since the hypoxia response is known to be controlled by FIH, we have determined that the hypoxia inducible factor 1 alpha (HIF1α) is activated in cells infected with oHSV, even in normal oxygen conditions. HIF1α is a transcription factor which activates a variety of genes in response to a lack of oxygen. We believe that HIF1α activation may be responsible for the increased vasculature of oHSV treated brain tumors. A screen of targetscan.org for herpes simplex virus 1 (HSV-1) miRNAs and their predicted target genes revealed multiple miRNAs predicted to target a protein called, factor inhibiting HIF1α (FIH). This protein functionally inhibits HIF1α activation by preventing the binding of HIF1α to DNA. We hypothesized that FIH would be negatively regulated by oHSV infected cells as well as HSV-1 infected cells. HSV-1 expresses two miRNA molecules, which target and down regulate FIH. Translation of miRNA inhibitors (antagomirs) was able to successfully abrogate HSV-1-induced downregulation of FIH, thus allowing HIF1α functionally inhibits HIF1α to DNA.

Submitted images (after USPIO minus before USPIO) of a tumor from an OV-treated rat (A) 15 minutes and (B) 30 minutes after USPIO administration. C) Immunofluorescence images of brain vasculature. Shown are brain, PBS-treated tumor tissue, and OV-infected tumor tissue relative to the level in contralateral normal untreated brain in each slice. D) Western blot of samples from each group. 

Inhibition of HSV miRNA rescues FIH down regulation

Figure 4. HSV induces down regulation of factor inhibiting HIF-1α (FIH)

A) LN229 cells were infected with increasing concentrations of oHSV in normoxia. RNA was isolated and analyzed by real time PCR for FIH expression. Relative FIH expression was compared to that of uninfected cells after 24 hours. B) LN229 cells were transfected with antagomirs that target HSV-1 miRNA-h16 and HSV-1 miRNA-h6. Cells treated with miRNA-h6 and miRNA-h16 displayed increased FIH expression compared to non-targeting control miRNA (NC). C) LN229-V6R cells were transfected with the reporter vector and infected with oHSV in normoxia. LacZ staining revealed increased HRE promoter activity following oHSV infection. D) HSV miRNA-H16 and HSV miRNA-H6 were predicted to target a protein called, factor inhibiting HIF1α (HSV-1) miRNAs and their predicted target genes revealed multiple miRNAs predicted to target a protein called, factor inhibiting HIF1α (FIH).

Figure 5. HSV expresses miRNA capable of targeting and down regulating FIH. A) LN229 cells were transfected with antagomirs specific to HSV miRNA-H16 and HSV miRNA-H6 and subsequently infected with oHSV. B) LN229 cells were transfected with PGL3 vector or PGL3 with dominant negative IkBa (constitutively active) and analyzed by real time PCR for HIF1α expression. 

Conclusions & Future Directions

Conclusions

1) Treatment with oHSV induces down regulation of the hypoxia response element promoter, and these results were consistent with the downregulation of angiogenes following oHSV therapy of brain tumors.

Figure 6. HSV miRNA activates NF-kB pathway.

A) U251T3 glioma cells were transfected with antagomirs specific to HSV miRNA-H16 and HSV miRNA-H6 and subsequently infected with oHSV. B) U251T3 glioma cells were transfected with PGL3 vector or PGL3 with dominant negative IkBa (constitutively active) and analyzed by real time PCR for HIF1α expression. 

Future Directions

1) Demonstrate that expression of miRNA is capable of activating the NF-kB promoter using the HER-B-Luc reporter vector.

2) Use antibodies to demonstrate increase of NF-kB activation following oHSV infection.

3) Identify the HSV-1 miRNA that is responsible for the observed effect.

4) Assess the effects of this HSV-1 miRNA in vivo. This next generation virus would have to be generated to specifically target the negative side effect of increased tumor vasculature.

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