Author's response to reviews

Title: Integrated microarray and multiplex cytokine analyses of Kaposi's Sarcoma Associated Herpesvirus viral FLICE Inhibitory Protein K13 affected genes and cytokines in human blood vascular endothelial cells

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Author's response to reviews: see over
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To

The Editor
BMC Medical Genomics

Re: Resubmission of 4804779123949398

Dear Editor:

Please find attached our revised manuscript entitled “Integrated microarray and multiplex cytokine analyses of Kaposi’s Sarcoma Associated Herpesvirus viral FLICE Inhibitory Protein K13-affected genes in human vascular endothelial cells” by Punj et al for consideration of publication in BMC Genomics. In the revised manuscript, we have addressed all major criticisms of both reviewers. Our point by point response to the reviewers follows:

Reviewer 1
1. A number of published studies have examined vFLIP and/or KSHV regulation of cellular gene expression in endothelial cells with some exclusively focusing on cytokines. The authors should compare their results with these studies, particularly for those that are different from their observations. To the minimum, these highly relevant published papers should be cited.

This has been done in the discussion section of the revised manuscript (page 12).

2. The authors state that the vFLIP HUVEC cannot be permanently maintained. How long were these cells infected. The expression of vFLIP was only induced for 48 h. Could the cells be still in transient state rather than static state as should be expected for latent KSHV-infected cells?

The K13-ER expressing cells were infected and selected in G418 to generate stable clones. The selection process took approximately 14 days. The cells were subsequently cultured for several passages (along with the control vector expressing cells) prior to being used in the experiment. Since K13 is a latent protein, its expression is relatively stable in KSHV infected cells as compared to the lytic genes that show a transient increase followed by a decline (Ref [1] and Additional Figure 5).

3. No information on the KSHV-infected cells was provided. How long they were
infected? What was the latent vs lytic status of the virus? This is very information that should be included since a number of viral lytic genes also regulate cellular cytokines.

We infected the HUVECs with KSHV for 48 h as a previous study had reported that the transient increase in lytic gene expression seen after infection returns to the baseline level by this time point [1]. We have confirmed these results by qRT-PCR analyses and this information is provided in Additional Figure 5, which shows that expression of lytic genes decline to near baseline level by 48 h after infection, while the expression of latent genes is still maintained.

5. vFLIP regulation of SOD2 has recently been reported (J Virol. 2009 Jan;83(2):598-611). The author should discuss and cite the paper. This has been done in the discussion section (page 14).

Reviewer 2
1. Authors should show western blot for some more up and down regulated proteins in HUVEC cells with K13 with and without 4OHT.

Immunoblot in Figure 2 was included to provide the initial validation of the microarray data at the protein level. A more comprehensive validation of the microarray data at the protein level was obtained by a multiplex cytokine assay, which compared the mRNA and protein level of 34 different genes (Table 3).

2. Fig 3. Western blot showing expressions of K13, K13 58AAA, MC159, IkB# and IkB# SS32/36AA in these reporter assays are needed.

This information is provided in the revised Figure 3.

3. It would be good to compare the expression profiles of some of the up- and down-regulated genes in KSHV infected cells to comment that these changes are specific to KSHV infection.

The effect of KSHV infection on the expression of cellular genes has been studied in the past [2] and, therefore, was not examined in the present study. In the present study, we have focused on the effect of 4OHT induced K13 activity on cellular genes and proteins expression. We demonstrate the specificity of the observed changes in gene expression by including 4OHT treated HUVECs-vector cells. In Table 3, we included supernatant from KSHV-infected cells to demonstrate that the cytokines/chemokines that are up- or down-regulated by K13 are similarly affected by KSHV infection. Since K13 is specific to KSHV and is not encoded by other viruses, the above results support the notion that the changes in gene/protein expression observed in our study are specific to KSHV. However, since in addition to KSHV, the NF-kB pathway is known to be activated by infection with a number of viruses, such as Epstein Barr Virus, we expect that at least some of the genes affected by KSHV will also be affected by infection with other viruses.

I thank the reviewers for their comments and suggestions and hope that you will find the revised manuscript suitable for publication in *BMC Medical Genomics*.

Thank you for your consideration.
Sincerely,

Preet Chaudhary, M.D., Ph.D.
Professor of Medicine

References
