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P120 - Human Herpesvirus 6A and 6B inhibit in vitro angiogenesis by induction of Human Leukocyte Antigen G

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1. Introduction

We have previously reported that human herpesvirus 6 (HHV-6) infection of endothelial cells (ECs) induces the loss of angiogenic properties, through the expression of HHV-6 U94, likely associated to the release of a soluble mediator.

On the other hand, the soluble isoform of HLA-G is known to exhibit an anti-angiogenic function, important in implantation, neoplastic and transplantation settings.

This study was therefore aimed to analyze the expression of HLA-G in HHV-6 infected ECs, to evidence any potential role in virus-induced anti-angiogenic activity.

2. Materials and methods

To evaluate HLA-G induction, human umbilical vein endothelial cells (HUVECs) were infected with HHV-6A or 6B, or nucleofected with plasmids expressing virus U94 gene, or treated with recombinant U94 protein. HLA-G induction was analyzed, by RT-PCR, flow cytometry and ELISA. Ability of virus genes to directly induce HLA-G expression was investigated by luciferase reporter assay. Induction of cell transcription factors was analyzed by RT-PCR microarray analysis. Angiogenesis ability was analyzed by capillary-like formation on basal membrane extract.

3. Results

Results showed that both HHV-6A and HHV-6B infection induce a potent up-modulation of HLA-G expression in infected ECs, including both membrane and soluble isoforms. Interestingly, HHV-6A and HHV-6B induced preferentially different isoforms of HLA-G. The virus-induced increase of HLA-G is likely due to the expression of the U94 virus gene, which reproduced the effect of whole virus. U94 effect is mediated by the induction of the human transcription factor ATF3, which recognizes a consensus sequence on the HLA-G promoter, inducing its activation.

Notably, the virus-induced inhibition of ECs angiogenic ability was directly correlated to HLA-G expression and release, since the addition of an anti-HLA-G antibody restored the angiogenic properties of HHV6-infected ECs.

4. Discussion and Conclusions

Our data show for the first time that HHV-6A and 6B infections induce up-modulation and release of HLA-G in human endothelial cells, and that this remodulation, and in particular the release of the soluble HLA-G isoform from infected cells, is directly related to the inhibition of angiogenic properties observed in ECs upon HHV-6 infection. Furthermore, the results indicates for the first time that virus infection induces ATF3, which is able to interact directly with the HLA-G promoter, finally inducing the HLA-G production associated to virus infection.

It will be interesting to analyze the virus effects also in different cell types, since these might be important in diverse clinical conditions involving not only the regulation of angiogenesis but also the development of immune response and inflammation.