Role of ERK1/2 protein in the regulation of *Herpes simplex* virus type I replicative cycle

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Introduction and Objectives

Herpes simplex virus type 1 (HSV-1) is a double stranded DNA (dsDNA) virus that causes a variety of infections in humans.[1] HSV-1, as many DNA viruses, has developed different strategies during the evolution to modify the cellular environment in favor of its replication. Different aspects of HSV-1 biology render this virus a good model to study the complexity of virus-host cell interactions. The eukaryotic cells, indeed, respond to external stimuli through the activation of different signal pathways, as the Ras/Raf/MEK/ERK signal pathway. Among these protein kinases, the extracellular-signal-regulated-kinases (ERK) have proven critical in the control of the progression G1/S that involved specific regulator proteins, such as cyclins and cyclin-dependent kinases (CDKs).[2,3] That HSV-1 infection requires involvement of ERK1/2 and mitogen activated proteins kinases (MAPKs) signal pathway and controls cell cycle proteins is already known.[4-9] In fact, the activity of CDK involved in the transition from late G1 to early S phase seems to be required for viral DNA transcription and replication. However, the evidence for the overall understanding of networks and gene products involved in these interaction requires further investigations. Based on these knowledge the current work was focused to study the activity of ERK1/2 protein during viral replication and the correlation between ERK protein recruitment and G1/S phases regulation by HSV-1 infection.

Materials and Methods

Western Blot analysis was used to evaluate nuclear and cytoplasmic protein accumulations. The MAPK/MEK-ERK pathway was modulated using the inhibitor U0126 to study replication in HEp-2 (human larynx epidermoid carcinoma cell line). A stably transfected cell line was derived from wild type (wt) HEp-2, by transfection of plasmid coding for dominant negative form of ERK protein (HEp-dnERK). Standard Plaque Assay was done on VERO cells. Immunofluorescence assay and quantitative Real Time PCR were used to evaluate the levels of viral and cellular gene transcription and viral DNA synthesis.

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Results and Discussion

We have analyzed the activation of ERK1/2 protein during wt HSV-1 infection in HEp-2 wt cell line. HSV-1 leads to the activation of ERK1/2 protein during the first phases of infection, and subsequent decrease during late phases compared to uninfected cells, suggesting involvement of ERK1/2 activity during infection. HSV-1 replication was studied in wt HEp-2 cells where ERK1/2 activity was chemically inhibited. The data showed a defect in viral progeny production in treated and infected cells as compared with non-treated and infected cells. These data were confirmed by the differences in the accumulation of ICP0 (immediate early) and Us11 (late) viral proteins. Moreover, we evaluated the phosphorylated forms of key regulators of G1/S progression, such as cyclin E and CDK2 proteins in presence or in absence of U0126. The results demonstrated that the treatment inhibits the accumulation of cyclin E and CDK2 proteins. These results were further confirmed by using HEp-dnERK cell lines. In this cell system HSV-1 replication was compromized compared with parental cell lines. Indeed, using q-PCR, viral DNA the cellular genes CDK2 and cyclin E, and the viral immediately early gene (ICP0) and the late gene, (gB) were evaluated in HEp-dnERK infected by wt HSV-1 compared to control. A decrease was observed in viral DNA synthesis, as well as in cellular gene transcripts, in cells where ERK1/2 activity was compromised, demonstrating that MAPK-ER proteins plays a fundamental role during HSV-1 replication. However, further investigations are necessary.

Conclusions

The new information obtained could be contribute to development of new pro-host tools that would be useful to set up effective prevention strategies, such as therapeudical approaches for severe HSV-associated infections. Because the Raf/Ras/MEK/ERK pathway is modified in 60% of solid tumors, understanding the interactions between HSV-1 and this pathway could contribute to the design of HSV-1-based oncolytic vectors.

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