IDENTIFICATION OF REPLICATION-COMPETENT HSV-1 CGAL$^+$ STRAIN SIGNALLING TARGETS IN HUMAN HEPATOMA CELLS BY FUNCTIONAL ORGANELLE PROTEOMICS

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In the present work, we have attempted a comprehensive analysis of cytosolic and microsomal proteomes to elucidate the signalling pathways impaired in human hepatoma cells (Huh7) upon Herpes Simplex Virus Type 1 (HSV-1 Cgal$^+$) infection. Using a combination of Differential in Gel Electrophoresis (DIGE) and nanoLC-MS/MS, 18 spots corresponding to 16 unique deregulated cellular proteins were unambiguously identified, which are involved in the regulation of essential processes such as apoptosis, mRNA processing, cellular structure and integrity, signal transduction and Endoplasmic-Reticulum Associated Degradation (ERAD) pathway. Based on our proteomic data and additional functional studies target proteins were identified indicating a late activation of apoptotic pathways in Huh7 cells upon HSV-1 Cgal$^+$ infection. Additionally to changes on RuvB-like 2 and Bif-1, down-regulation of Erlin-2 suggests stimulation of Ca^{2+}-dependent apoptosis. Moreover, activation of the mitochondrial apoptotic pathway results from a time dependent multi-factorial impairment as inferred from the stepwise characterization of constitutive pro- and antiapoptotic factors. Activation of Serine-threonine protein phosphatase 2A (PP2A) was also found in Huh7 cells upon HSV-1 Cgal$^+$ infection. In addition, PP2A activation paralleled dephosphorylation and inactivation of downstream MAP kinase pathway (MEK1/2, ERK1/2) critical to cell survival, and activation of proapoptotic Bad by dephosphorylation of Ser112. Taken together, our results provide novel molecular information that contributes to define in detail the apoptotic mechanisms triggered by HSV-1 Cgal$^+$ in the host cell and lead to the implication of PP2A in the transduction of cell death signals and cell survival pathway arrest.