QUAKING RNA BINDING PROTEINS AS MEDIATORS OF ONCOLYTIC HSV VECTORS IN HUMAN HEPATOMA CELLS

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The poor prognosis of HCC patients makes necessary the development of novel and more efficient strategies. Oncolytic viral therapy using HSV-1-based vectors stand out as a treatment of liver tumours. Little is known about the cellular targets that are modulated during viral infection. We have analysed the molecular mechanisms mediating the effects of HSV-1 $Cgal^+$ infection in hepatoma cell nuclei using two complementary proteomic techniques. Human hepatoma Huh7 cells were infected with HSV-1 $Cgal^+$ oncolytic viruses at MOI of 5 pfu/cells during 8h. Nuclear fractions were analysed using two different proteomic methods: 2D-DIGE and tandem mass tag (TMT) peptide labelling and mass spectrometry. Further validations were performed by Western blot, immunofluorescence microscopy and siRNA silencing.2D-DIGE analysis revealed expression changes in 24 unique proteins meanwhile TMT showed 26 differentially expressed proteins. Although both sets of proteins are different, the two proteomic techniques are complementary and are involved in the same cellular processes such as host-virus interaction, transcription regulation, mRNA processing and mRNA splicing. Among the differentially expressed proteins, we identified an increase in Quaking RNA Binding protein (QKI) further validated by Western blot. We demonstrated a time-dependent increase of QKI levels, showing a peak at 4hpi together with the start of the expression of immediate-early viral proteins such as ICP27 or ICP4. Additionally, we proved that QKI-5 remains functionally active as an increase of one of its targets, $p27^{Kip1}$, was detected. Together with an increased $p57^{Kip2}$ expression, these data suggest an early cell cycle arrest after HSV-1 infection, as they both act as negative regulators of the G1-S transition. Finally, a nuclear-cytosolic shuttling of QKI-5 was demonstrated upon HSV1 infection. QKI-5 tends to localize in cell-to-cell contact regions 16 and 24hpi, showing a wrinkled line sort of pattern.