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Abstract (poster session)

Use of MALDI-TOF mass spectrometry for analysis of virus-infected cells: a preliminary report

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Objectives: The diagnosis of viral infection traditionally relies on direct methods based on cell culture, antigen or nucleic acid detection. This study aimed to demonstrate the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) ability in detecting specific biomarkers to discriminate between uninfected and virus-infected cells, not yet investigated for diagnostic purposes. Methods: Confluent Intestine 407 cells (ATCC CCL-6), grown for 48h in a 24-well plate, were inoculated with Adenovirus (ADV) (NIAID). After a 45-minutes absorption, the viral inoculum was replaced with a maintenance medium and infected cells were incubated at 37°C. At different times post-infection (p.i.), the uninfected and infected cells were washed twice with PBS, harvested in 300 µ l of distilled water and the proteins were extracted following the manufacturer's protocol. After 72 h p.i. ADV purified particles from cultures were subjected to the same extraction protocol. Finally, 1 uL of all the protein extractions was transferred into the target plate and matrix (satured solution of a cyano-4-hydroxicinnamic acid in 50% acetonitrile) was added, followed by crystallization and air-drying. Spectra from samples analized by Microflex LT mass spectrometer (Bruker Daltonics) were recorded in positive linear mode within a mass range from m/z 2 to 20 KDa. Results: Uninfected and virus-infected cells were examined at 48 and 72 h p.i.. A rate of purified ADV was also analyzed. The spectra obtained from the analysis of uninfected cells were used as the baseline for the detection of any significant protein composition change into the ADV-infected cells, resulting from the inhibitory effect of the viral infection on the cellular protein synthesis or from the synthesis of viral specific proteins. The spectra obtained from ADV-infected cells showed two significant peaks which are completely missing in the spectra of uninfected cells and overlapping those obtained by the analysis of purified viral particles. Moreover, most of the peaks which appeared in control uninfected cells spectra completely disappeared in the infected cells. Conclusion: Although the results obtained in this study are preliminary and should be confirmed using also different virus-cells models, the spectral differences observed between uninfected and virus-infected cells may be a promising basis for the spectroscopic detection and identification of infected cells with different viruses in clinical virology.