PROTEOMIC CHARACTERIZATION OF INFLUENZA H1N1 GAG VIRUS-LIKE PARTICLES AND EXTRACELLULAR VESICLES PRODUCED IN HEK-293SF

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One of the major concerns associated with the use of influenza virus-like particles (VLPs) produced in cell culture as vaccine candidates is their heterogeneous composition. Enveloped VLPs take up the host cell membrane at the budding site carrying out with them not only the viral antigenic proteins but also host cell proteins. In addition, the intrinsic nature of the cells to produce membrane derived vesicles which have similar size to the VLPs and can also contain the antigenic proteins, makes the VLP purification process challenging. Certainly, the expression system and the viral recombinant proteins employed will determine the nature of the proteins within the VLPs. To further characterize cell culture produced-influenza VLPs and contribute to enable their approval as vaccine candidates, the composition and biogenesis of VLPs need to be better understood. In this study we have characterized, by nanoscale liquid chromatography tandem mass spectrometry (n-LC-MS/MS), influenza H1N1 Gag-VLPs produced in human embryonic kidney cells adapted to serum-free medium (HEK-293SF). The cells stably express HA and NA, and the VLPs production occurs following transient transfection with a plasmid containing the gag gene of HIV-1 fused to GFP. Extracellular vesicles (EVs) produced by the unmodified HEK-293SF were also characterized by n-LC-MS/MS. A total of 73 host cell proteins were identified in the VLPs, whereas 98 were detected in the extracellular vesicles. From that, 32 host cell proteins were unique to VLPs while 41 proteins were found in both. Importantly, nucleolin was the most abundant host cell differential protein identified in VLPs while lactotransferrin and heat shock protein 90 were the most present in EVs. This study provides a detailed proteomic description of the VLPs and EVs produced in HEK-293SF as well as a critical discussion of the function of each protein incorporated in both nanoparticles species. The outcome of this research also sheds light on unique target proteins differentially identified either in VLPs and EVs that could potentially be exploited for the development of novel purification protocols to separate EVs from VLPs.