Morphological and proteomic characterization of exosomes released from influenza A virus infected ECV cells

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Intercellular communications are generally realized through the release of protein molecules, which are able to bind specific receptors on the surface of the surrounding cells. However, cells additionally secrete more complex structures, called membrane vesicles [1]. Exosomes are cell-derived membrane vesicles formed from the multivesicular bodies. Their diameter varies from 30 to 100 nm, the density ranges between 1.13 and 1.19 g/ml [2]. More than 2000 proteins, about 900 RNA and 274 microRNA molecules were found in exosomes, released by different cell types [3]. The biochemical structure and biological properties of exosomes depend on the place of their formation [4]. It is now assumed, that viruses use microvesicles formation pathways for assembly and budding, evasion of the immune response and cell-cell communications [5]. Therefore, it seems possible, that exosomes are able to play an important role in the life cycle of influenza viruses.

The purpose of the current research is to carry out a molecular biological characterization of exosomes, released from influenza virus infected and mock infected ECV cells. In particular, we have to develop the protocol of separation, purification and characterization of exosomes, released from influenza A virus infected and mock infected cells, and to compare the alterations in protein composition of exosomes in response to influenza virus infection.

Exosome fractions were isolated from mock infected ECV cells, using the centrifugation in sucrose gradient method. Exosome fractions were characterized on their size and morphology by electron microscopy and atomic force microscopy. Western-blotting analysis, using monoclonal antibodies to exosome marker proteins (annexin A2, CD9, CD63, HLA-ABC, HSP70), was made for exosome fractions. We have chosen the fractions, which corresponded to exosomes by the size, shape and marker proteins composition. The same procedures were repeated to analyze the exosomes from the infected cells. However, it was shown that influenza virus particles are isolated in the same fractions as exosomes. Thereby, the separation of influenza virus particles and exosomes has to be done.

At the moment we have made the protocol of isolation of the exosomes, derived from mock infected ECV-cells. After the separation of exosome proteins using SDS-PAGE, followed by silver staining, each lane was cut into sequential slices from top to bottom and the individual slices were subjected to in-gel trypsin digestion. The resulting spectra of tryptic peptides, obtained on MALDI-TOF/TOF mass spectrometer, are now being processed.

Exosomes were isolated from mock infected ECV-cells culture media and were characterized. The separation technique of the influenza virus particles and exosomes is in development.

References.

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