Subcellular Processes in Plants and Animal Cells

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Characterization of HBV transport pathways in macrophages

X. Cheng[1,2], J. Wettengel[1], K. Esser[1,2], U. Protzer[1,2]

[1]Institute of Virology, Technische Universität München , Munich, Germany
[2]Institute of Virology, Helmholtz Zentrum München, Munich, Germany

xiaoming.cheng@virologie.med.tum.de

The human hepatitis B virus (HBV) is a DNA virus with high species specificity and liver tropism. Our preliminary studies suggest that in human liver HBV is initially sequestered by Kupffer cells, which represent the residential macrophages, and subsequently transcytosed towards its host cell, the hepatocyte. This study aims at identifying the transportation route of HBV within Kupffer cells.

To trace the intracellular transportation of HBV, fluorescence labelled viral particles (VP) were produced. VP were harvested from HBV producing HepG2.215 cells, purified by different density gradient centrifugation and chemically labelled with an Alexa Fluor® dye. Successful labelling of viral surface proteins was confirmed by immunoassays and fluorescence analysis of protein gels. Detection of labelled VP in monocyte derived macrophage (MDM) using confocal microscopy found that after binding to MDM, VP first enter into early endosomes but were not targeted to lysosome in the later time point. Filipin was used to label free cholesterol in the cell, which was shown to localize within identical organelles as VP, suggesting a linked transport of both components. To further characterize VP transportation, intracellular cholesterol transport was blocked by a specific inhibitor U18666a and it was found that VP recycling to the plasma membrane was inhibited at the same time. To further analyse compartmentalization of HBV in macrophages, cholesterol transporting protein NPC1 was labelled by indirect immunofluorescent staining and was shown to be colocalized with VP. Finally, associated transportation of HBV with lipoprotein derived free cholesterol could be confirmed in Kupffer cells by colocalization of HBV with NBD-cholesterol originated from NBD-cholesterol labelled triglyceride rich lipoproteins.

Taken together, we propose that HBV highjacks the free cholesterol transport machinery in monocyte derived macrophages and Kupffer cells to escape lysosomal degradation and to become cellularly transcytosed which lead to trans-infection of the host cell.