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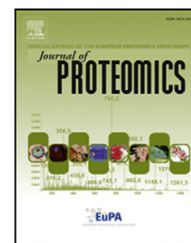

Proteomic analysis identifies a novel function for galectin-3 in the cell entry of parvovirus

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ABSTRACT

Cellular factors associated with the parvovirus minute virus of mice (MVM) during infection are thought to play important roles in the MVM life cycle but only a few of these have been identified. Here we used a proteomic-based approach in order to identify host-binding partners of MVM. Using purified MVM as bait for immunoprecipitation assays, a total of 150 proteins were identified in MVM immunoprecipitates by quantitative liquid chromatography–tandem mass spectrometry. Galectin-3 was one of six proteins showing a statistically significant enrichment across replicates. Small interfering RNA depletion studies revealed an important role for galectin-3 in MVM endocytosis and infectivity in LA9 mouse fibroblast cells. Galectin-3-depleted cells were less susceptible to MVM infection than control cells and showed a significant reduction of MVM cellular uptake, but not of MVM binding to the cell surface. Our results indicate an important role for galectin-3 in the cellular uptake of MVM. We propose that galectin-3 facilitates the access of MVM to its receptor(s) at the plasma membrane and in this way promotes MVM endocytosis.

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1. Introduction

The minute virus of mice (MVM) is a small, nonenveloped, single-stranded DNA virus that belongs to the *Parvoviridae* family. MVM is among the smallest DNA animal viruses with a ~5 kb genome containing only two open reading frames, coding for two non-structural proteins and two capsid proteins [1]. Because of their very simple genome and their ability to preferentially replicate in cancer cells, MVM and other rodent parvoviruses have attracted considerable attention for potential use in cancer gene therapy [2–5]. Thus, extensive studies have characterized the structure and organization of the viral capsid and its genome, the viral replication and transcription, the virus-cell interaction and the pathogenicity of MVM (reviewed in [6,7]). Despite this progress, not all the steps of the lifecycle of MVM have been completely characterized.

The ~5 kb genome of MVM is enclosed within an icosahedral capsid of 26 nm in diameter, which is composed of 60 copies of three capsid proteins, designated VP1 (83 kDa), VP2 (64 kDa), and VP3 (60 kDa) [8,9]. While enveloped viruses employ a

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repertoire of membrane and accessory proteins to enter their host cells, parvoviruses rely only on the capsid's proteins to perform both cell entry and a wide variety of biological functions during the viral life cycle. The MVM capsid first recognizes and binds to sialic acid structures of a not-yet identified host cell surface receptor(s) [10]. After being taken up into the host cell by receptor-mediate endocytosis using most likely clathrin-coated vesicles [11], the capsid slowly escapes from the endocytic compartments to the cytosol using phospholipase A2 (PLA2) enzymatic activity in the capsid protein VP1 [12]. This is followed by the release of the capsid into the cytoplasm, which may interact with the cytoskeleton and molecular motors for intracellular trafficking. The capsid is then imported into the nucleus by transiently disrupting the nuclear membranes in a caspase-dependent manner [13]. In the nucleus, the capsid releases the viral DNA, which then waits for the host cell to transition into the S-phase, in order to initiate viral DNA synthesis using the cellular replication machinery [14].

At every step of the infectious cycle of MVM described above, the MVM capsid interacts with multiple host cellular factors. However, very few cellular interaction partners had been previously identified. To identify novel cellular binding partners of MVM we performed a proteomic analysis of proteins from a cell lysate that co-precipitated with purified MVM. Our analysis identified at least six putative MVM binding proteins, some of which have been previously identified or predicted to interact with MVM. Two of the proteins identified were the soluble extracellular matrix protein galectin-3 (Gal-3) and its binding protein Gal-3BP. Gal-3 is a member of the β-galactoside-binding lectin family, defined by conserved peptide sequence elements involved in carbohydrate binding activity [15]. Since there is very little information concerning the roles of galectins in viral infection, except for Gal-1 which promotes the infectivity of HIV-1 and Nipah virus [16–19], here we characterize the contribution of Gal-3 to MVM infection. Our results revealed that Gal-3 is involved in the endocytic pathway of MVM.

2. Materials and methods

2.1. Cells, virus and antibodies

LA9 mouse fibroblast cells were maintained at 5% CO_2 and 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% glutamine and penicillin–streptomycin. MVM was propagated in LA9 cells grown in suspension, and purified based on the protocols described previously [20,21].

The antibody against intact MVM capsid (MAb B7, [22]) was generated in D4H1 mouse hybridoma cells (kindly provided by Dr. P. Tattersall, Yale University School of Medicine). The mouse monoclonal antibody specific for the C-terminus of MVM non-structural protein 1 (NS1) [23] was a gift from Dr. Tattersall. The polyclonal rabbit anti-galectin-3 antibody (H-160) was purchased from Santa Cruz Biotechnology. Fluorescein isothiocyanate (FITC) labeled-phalloidin and fluorescently labeled secondary antibodies were purchased from Invitrogen. Mouse monoclonal antibody [AC-15] to beta actin (AC-15) was from Abcam.

2.2. Immunoprecipitation

Antibody against intact MVM capsids (MAb B7) was first coupled to NHS-activated sepharose beads (GE Healthcare) according to the manufacturer's instructions. For immunoprecipitation of MVM, sepharose beads coupled with 60 μg of antibody were incubated or mock incubated with 4 μg purified MVM, overnight at 4 °C while rocking. Beads were then washed three times with lysis buffer (150 mM NaCl, 50 mM Tris, 1 mM EDTA, 1% NP40, pH 7.2).

LA9 lysates were harvested in lysis buffer containing protease inhibitor mixture (Roche Applied Science) and cleared by overnight incubation at 4 °C with inactivated sepharose beads (in the absence of antibody). The MVM- and mock-incubated beads were each incubated with 10 mg of pre-cleared cell lysate, overnight at 4 °C. This was followed by four washes with lysis buffer and two washes with PBS. After the final wash, all remaining buffer was drawn from the beads until the beads were dried, and bound protein was eluted by incubation with 30 μl of Laemmli sample buffer at 96 °C for 5 min.

2.3. Proteomics

Proteins eluted from four independent biological replicates of mock and MVM immunoprecipitates were electropheresed, in separate lanes, approximately 3 mm into a 10% SDS-PAGE and visualized with colloidal Coomassie staining [24]. For each sample the entire stained area of the gel was excised and digested exactly as described [25]. Mock and MVM samples were differentially labeled with formaldehyde isotopologs, as described [26], before being combined and analyzed on an Orbitrap-XL (ThermoFisher) exactly as described [25]. Fragment spectra were searched against the mouse International Protein Index database (v3.78) with reversed sequences, common contaminants and MVM sequences concatenated (110,856 sequences) using Mascot (v2.2, Matrix Science). The MSQuant package [27] was then used to extract quantitative data and manually inspect extracted ion chromatograms. Proteins were considered identified if at least two peptides were detected with Ions Scores above 28, giving an estimated false discovery rate of $<1\%$ based on matches against the reversed sequences. To determine which proteins were significantly enriched in MVM vs. mock immunoprecipitates a one-tailed z-test was applied to test the hypothesis that each ratio was not greater than 1.0, the ratio expected if there were no enrichment. Only those proteins where ratios were measured in at least two of the four replicates and where $p<.01$ were considered enriched. To compensate for type 2 errors resulting from multiple hypothesis testing, the Bonferroni correction was applied to each p-value.

2.4. siRNA

LA9 cells were either mock transfected with Lipofectamine RNAiMAX (Invitrogen) or transfected with previously described siRNA oligonucleotide sequences targeting Gal-3 [28] accordingly to the manufacturer's recommendations. A pool of four non-targeting siRNAs (ON-TARGETplus Non-Targeting Pool, Dharmacon) was used as control siRNA. Expression of

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