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Galectin-3 plays a role in minute virus of mice infection

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ABSTRACT

Galectin-3 has previously been found to be required by the parvovirus minute virus of mice prototype strain (MVMp) for infection of mouse fibroblast cells. Since MVMp is an oncotropic virus, and galectin-3 is a multifunctional protein implicated in cancer metastasis, we hypothesized that galectin-3 and Mgat5, the Golgi enzyme that synthesizes high-affinity glycan ligands of galectin-3, might play a role in MVMp infection. Using siRNA-mediated knockdown of galectin-3 in mouse cells transformed with polyoma-virus middle T antigen and Mgat5$^{-/-}$ mouse mammary tumor cells, we found that galectin-3 and Mgat5 are both necessary for efficient MVMp cell entry and infection, but not for cell binding. Moreover, we found that human cancer cells expressing higher levels of galectin-3 were more efficiently infected with MVMp than cell lines expressing lower galectin-3 levels. We conclude that galectin-3 and Mgat5 are involved in MVMp infection, and propose that galectin-3 is a determinant of MVMp oncotropism.

Introduction

Galectin-3 (Gal-3) is a member of the β-galactoside-binding lectin family of proteins, which bind to carbohydrates (reviewed by Boscher and Nabi, 2013; Vasta, 2012). Specifically, Gal-3 binds to the β1,6 branched N-glycans of glycoproteins on the cell surface, which are the products of the Golgi enzyme β1,6-acetylglucosaminyltransferase 5 (Mgat5) (reviewed by Boscher et al., 2011). On the cell surface, Gal-3 interacts with several ligands, including integrins and epidermal-growth factor receptor (EGFR) (reviewed by Boscher et al., 2011). Therefore, it has been proposed that Gal-3 modulates the clustering and signaling activity of receptors on the plasma membrane (Boscher et al., 2011; Goetz, 2009). Similarly, through its interactions with Mgat5 modified N-glycans of various cell surface receptors and proteins, Gal-3 modulates several cellular functions, such as signal transduction on the cell surface and cell adhesion, motility, growth, and differentiation (Dennis et al., 2009b). Since some of these functions are altered in tumorigenic cells, Gal-3 regulates many physiological and pathological cellular processes. However, a role for Gal-3 in viral infection has only recently been discovered. In a proteomic study, Gal-3 and its binding protein, Gal-3-binding protein (Gal-3-BP), were identified as cellular partners of the parvovirus minute virus of mice prototype strain (MVMp) (Garcin et al., 2013). In addition, a screen for serum proteins that interact with different adeno-associated virus (AAV; another parvovirus) types identified Gal-3-BP as a binding partner of AAV (Denard et al., 2012). It was subsequently shown that Gal-3 is required for efficient MVMp cellular uptake and infection, but not for viral binding to the plasma membrane (Garcin et al., 2013), and that Gal-3-BP reduces AAV-6 transduction (Denard et al., 2012). In addition to these parvoviruses, it has also been reported that herpes simplex virus type 1 (HSV-1) uses extracellular Gal-3 for cell entry (Woodward et al., 2013).

MVM is a small (26 nm in diameter), non-enveloped virus with a single-stranded DNA genome that belongs to the *Parvoviridae* family (Cotmore et al., 2014). The MVM genome is protected by a capsid assembled from 60 copies of two size variants of the capsid proteins VP1 and VP2, which are identical, except for the unique N-terminal sequence (140 amino acids) of VP1 (Tattersall et al., 1977). Several strains of MVM exist, but the best characterized strains are the prototype MVMp that infects cells of fibroblast origin and the immunosuppressive strain MVMi that infects T lymphocytes (Tattersall and Bratton, 1983). This MVM tropism is controlled predominantly by the capsid protein VP2, with only two amino acid substitutions on this protein making the virus fibrotropic or lymphotropic (Ball-Goodrich and Tattersall, 1992).

Several parvoviruses attach to their target cells via binding of capsid proteins to glycan receptors (reviewed by Huang et al., 2014). For example, human parvovirus B19 uses gangliosides (Cooling et al., 1995), AAV2 uses heparan sulfate proteoglycan (Kern et al., 2003), and rat H-1 parvovirus, porcine parvovirus, and MVM use sialic acid (Allaume et al., 2012; Boisvert et al., 2010; Nam et al., 2006). A recent sialylated glycan microarray revealed that MVM binds to diverse sialylated derivatives, but there was different recognition between MVMp and MVMi (Halder et al., 2014). For example, MVMp, but not

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MVMi, binds to 9-*O*-acetylated and 9-*O*-lactoylated sialic acid derivatives (Halder et al., 2014). Despite this difference, MVM attachment to sialic acid induces virion internalization by clathrin-dependent endocytosis (Linser et al., 1977, 1979; Vihinen-Ranta and Parrish, 2006) (reviewed by Vihinen-Ranta and Parrish, 2006), and possibly other endocytic pathways as has been recently shown for other parvoviruses (Bantel-Schaal et al., 2009; Boisvert et al., 2010; Nonnenmacher and Weber, 2011). Following endocytosis, MVM escapes from endocytic compartments into the cytosol by means of the enzymatic action of a phospholipase A2 (PLA2) domain in the unique region of VP1 (Farr et al., 2005). The virions that escape the endosomes then enter the nucleus where they wait for the host cell to transition into the S-phase, to initiate viral DNA replication (Cotmore and Tattersall, 2006).

MVMp and other rodent parvoviruses have several properties that make them good candidates for development as cancer therapies (reviewed by Nuesch et al., 2012; Rommelaere et al., 2010). In fact, there is an ongoing clinical trial using the rat parvovirus H-1 in patients with recurrent glioblastoma (Geletneky et al., 2012). For MVMp, it is well established that in vitro this virus preferentially infects human tumor cells that are stably transformed or in which transformation has been induced (Legrand et al., 1993; Mousset et al., 1986; Wollmann et al., 2005; Zeicher et al., 2003). The molecular bases of this MVMp oncotropism are not completely understood. Nevertheless, several parameters have been associated with MVMp oncotropism. These include the S-phase dependence of viral replication (Burnett and Tattersall, 2003; Deleu et al., 1999), the MVMp sensitivity to the antiviral response (Grekova et al., 2010; Mattei et al., 2013), Raf-1-dependent phosphorylation of the viral structural protein VP2 (Riolobos et al., 2010), post-translational modifications of the viral non-structural protein NS1 (Nuesch et al., 2003), and the process of epithelial–mesenchymal transition (Garcin and Pante, 2014).

Since Gal-3 has been implicated in cancer progression (Miranda et al., 2009; Mourad-Zeidan et al., 2008; Takenaka et al., 2004; Wang et al., 2013, 2009) and is considered as a potential marker for aggressive tumor cells (Chiu et al., 2010; Shankar et al., 2012), we hypothesized that Gal-3 and Mgat5 might be involved in MVMp infection. To test this hypothesis, we characterized the role of Gal-3 and Mgat5 in the infectious cycle of MVMp. Using small interfering ribonucleic acid (siRNA) to silence Gal-3 expression and cells derived from Mgat5 knockout mice (Mgat5$^{-/-}$ cells Granovsky et al., 2000), we found that Gal-3 and Mgat5 are required for efficient MVMp uptake and infection. In a screen of human cancer cells from various tissues, we also found a correlation between the Gal-3 expression profile of these cells and their susceptibility to MVMp infection, indicating that Gal-3 could be involved in MVMp oncotropism. Taken together, our results show that Gal-3 and Mgat5 are necessary for MVMp uptake and infection, and we propose Gal-3 as a potential regulator of MVMp oncotropism.

Results

Galectin-3 knockdown hampers MVMp infection in PyMT cells

It has been reported that Gal-3 is involved in MVMp uptake and infection of LA9 mouse fibroblast cells (Garcin et al., 2013). Therefore, we first confirmed this observation in a different cellular model derived from PyMT-transformed mouse mammary epithelial cells (Granovsky et al., 2000). These cells have been used to study the various cellular functions of Gal-3, such as its role in cell migration (Goetz et al., 2008). We first performed siRNA-mediated Gal-3 knockdown (KD) experiments in PyMT cells, and confirmed Gal-3 KD by immunofluorescence (IF) microscopy and Western blot analysis, which showed that Gal-3 levels were significantly reduced at 48 h post-transfection (Fig. 1A and B). These cells were then infected with MVMp at an MOI of 4 for 24 h at 37 °C, and then prepared for MVMp infection analysis by IF microscopy. The viral protein NS1 (which is used for measuring MVMp replication and infection since it is the first viral protein that is expressed upon successful MVM infection and it initiates viral DNA replication Doerig et al., 1990, 1988) and Gal-3 were detected using specific antibodies. As shown in Fig. 1C and D, the percentage of NS1-expressing cells was lower in cells treated with Gal-3 siRNA than in those treated with Lipofectamine or a non-targeting control siRNA. These results indicate that Gal-3 is required for efficient MVMp infection of PyMT cells, in agreement with previously published data in LA9 cells (Garcin et al., 2013).

Galectin-3 knockdown reduces MVMp uptake by PyMT cells

Next, we analyzed the effect of Gal-3 KD on MVMp cell entry by IF microscopy. PyMT cells were transfected with Gal-3 siRNA, a control siRNA, or Lipofectamine, and subsequently infected with MVMp at an MOI of 4 for 2 h at 37 °C in the presence of bafilomycin A1 (bafA1). BafA1 inhibits the vacuolar H^+-ATPase in the endosomal membrane that is responsible for acidification (Bayer et al., 1998); thus, MVMp arrests in early endosomes, which allows for better observation of the virions that enter the cells. As shown in Fig. 2, MVMp uptake was significantly lower in PyMT cells treated with Gal-3 siRNA than in control cells. This indicates that, similar to the results observed in LA9 cells (Garcin et al., 2013), Gal-3 promotes MVMp uptake in PyMT cells.

MVMp does not infect Mgat5$^{-/-}$ cells

It is well known that Mgat5 overexpression in cancer cells promotes tumor progression and invasion in a Gal-3-dependent manner (reviewed by Dennis et al., 2009b). Hence, we next tested the possible role of Mgat5 in MVMp infection using PyMT mouse epithelial mammary tumor cells derived from Mgat5 knockout mice (Mgat5$^{-/-}$ cells, Granovsky et al., 2000). Total lysates of LA9, PyMT, Mgat5$^{-/-}$, and Mgat5-rescued (Mgat5$^{-/-}$ cells stably transfected with a retroviral Mgat5 expressing vector, Partridge et al., 2004) cells were blotted and first probed for Mgat5-modified N-glycosylation using *Phaseolus vulgaris* lectin-coupled horseradish peroxidase (L-PHA-HRP) to verify that the Mgat5$^{-/-}$ cells lack these glycosylations. As shown in Fig. S1, in contrast to LA9 and PyMT cells, Mgat5$^{-/-}$ cells were indeed deficient in Mgat5-modified N-glycans. Moreover, Mgat5-rescued cells displayed much higher levels of Mgat5 expression than LA9 and PyMT cells.

These cells were then infected with MVMp at an MOI of 8 for 24 h at 37 °C and prepared for IF microscopy to detect both the viral NS1 protein and the newly produced viral particles that should have already been generated at 24 h post-infection. MVMp capsids and NS1 protein were detected with specific antibodies. As shown in Fig. 3, approximately 80% of the LA9 and PyMT cells were positive for NS1 and MVMp progeny. Strikingly, Mgat5$^{-/-}$ cells showed neither NS1 expression nor progeny virion production, indicating that these cells are resistant to MVMp infection. This phenotype was reversed in Mgat5-rescued cells, which were as permissive to MVMp infection as the wild-type PyMT cells (Fig. 3). These findings were validated by measuring NS1 expression levels in cell lysates from all four cell lines after 24 h of infection with MVMp at an MOI of 8. As shown in Fig. 4A, no NS1 was detected in Mgat5$^{-/-}$ cells, whereas LA9, PyMT, and Mgat5-rescued cells showed comparable amounts of NS1.

To further test the resistance of Mgat5$^{-/-}$ cells to MVMp infection, we performed plaque assays with MVMp and all four cell lines. In this experiment, the cells were infected with various concentrations of virus for 4 days, and then, virus-induced cell lysis was observed as the formation of plaques. As shown in Fig. 4B, Mgat5$^{-/-}$ cells were resistant to MVMp infection even at higher viral concentrations, while all the other cell types showed plaques even at a virus dilution