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Cell migration is another player of the minute virus of mice infection

Pierre O. Garcin, Nelly Panté *

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, Canada V6T 1Z4

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ABSTRACT

The parvovirus minute virus of mice, prototype strain (MVMp), preferentially infects and kills cancer cells. This intrinsic MVMp oncotropism may depend in part on the early stages of MVMp infection. To test this hypothesis, we investigated the early events of MVMp infection in mouse LA9 fibroblasts and a highly invasive mouse mammary tumor cell line derived from polyomavirus middle T antigen-mediated transformation. Using a combination of fluorescence and electron microscopy, we found that various parameters of the cell migration process affect MVMp infection. We show that, after binding to the plasma membrane, MVMp particles rapidly cluster at the leading edge of migrating cells, which exhibit higher levels of MVMp uptake than non-motile cells. Moreover, promoting cell migration on a fibronectin matrix increased MVMp infection, and induction of epithelial–mesenchymal transition allowed MVMp replication in non-permissive epithelial cells. Hence, we propose that cell migration influences the early stages of MVMp infection.

Introduction

The minute virus of mice (MVM) is a small (26 nm in diameter), non-enveloped, single-stranded DNA virus that belongs to the family *Parvoviridae* (Cotmore et al., 2014). The MVM genome of 5-kb carries only two open reading frames: the "right" (5′) codes for the two capsid proteins VP1 and VP2, while the "left" (3′) codes for the two non-structural proteins, NS1 and NS2 (Berns, 1990). MVM exists in two variant forms: MVMp, which is the prototype strain (Crawford, 1966), infects cells of fibroblast origin, and MVMi, the immunosuppressive strain, infects T lymphocytes (Bonnard et al., 1976). MVMp, unlike MVMi, is harmless to adult mice, and selectively infects cancer cells (Mousset and Rommelaere, 1982; Rommelaere et al., 2010). This property of MVMp, also known as oncotropism/oncolytism, together with the apathogenicity of MVMp to humans, has presented the exciting possibility of using MVMp in anti-cancer therapy.

Several studies have attempted to explain why MVMp preferentially targets rapidly dividing cancer cells (reviewed by Nuesch et al. (2012)), and several determinants for MVMp oncotropism have been proposed. First, because MVMp replication depends on host cell factors that are present during the S phase of the cell cycle (reviewed by Cotmore and Tattersall (2006)), the viral DNA replicates more easily in rapidly dividing cancer cells, which escape growth control. For example, conversion of the viral single-stranded DNA to double-stranded DNA depends on cyclin A and cyclin A-associated kinase activity (Bashir et al., 2000); subsequent transcription of the double-stranded DNA depends on the host transcription factors E2F and CREB/ATF (Burnett and Tattersall, 2003; Deleu et al., 1999; Paglino et al., 2007), which become available during the S-phase and which are also deregulated in cancer. Second, MVMp oncotropism has also been attributed to the posttranslational modification of parvoviral and cellular proteins by cellular kinases that are overexpressed or altered in cancer cells. For example, trimers of the capsid protein VP2 undergo phosphorylation by Raf-1 kinase (Riolobos et al., 2010); this phosphorylation regulates the nuclear transport of the VP2 trimers for subsequent assembly of progeny viral capsids in the nucleus (Riolobos et al., 2010). Similarly, phosphorylation of NS1, which controls the activity of this protein during both viral DNA replication and transcription, and phosphorylation of the eukaryotic translation initiation factor 2 subunit 1 (eIF2α) by protein kinase R have been proposed to contribute to MVMp oncotropism (Nuesch et al., 2012; Ventoso et al., 2010). Finally, it has been proposed that the failure of cancer cells to mount an antiviral response mechanism contributes in part to MVMp oncotropism. This is supported by the finding that MVMp infection of normal, but not transformed, mouse embryonic fibroblasts induces the production of type I interferons (IFNs) (Grekova et al., 2010; Mattei et al., 2013). However, MVMp replication is unaffected by MVMp-induced IFNs or treatment of cells with IFNs (Mattei et al., 2013; Paglino et al., 2014). Moreover, the MVMp-induced activation of the type I IFN anti-viral response is cell-dependent, and does not occur in human normal or cancer cells

* Corresponding author. Fax: +1 604 822 2416.
E-mail address: pante@zoology.ubc.ca (N. Panté).

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(Paglino et al., 2014). Thus, the involvement of the IFN anti-viral response in MVMp oncotropism has not been well established.

All these studies have indicated that MVMp oncotropism is mediated on several levels of the viral infection cycle. However, to date there has been no indication of whether the molecular mechanisms regulating MVMp oncotropism could also be regulated at earlier stages during the MVMp infection cycle. Since several types of aggressive tumor cells exhibit increased migration, which depends on the uptake and degradation of cell surface receptors (Nagano et al., 2012), we hypothesized that MVMp, and other oncotropic viruses, take advantage of some aspects of the cell migration machinery in order to preferentially infect highly metastatic/invasive cancer cells. In support of this hypothesis, the cells commonly used for studies of MVMp infection and cell migration are fibroblasts. Furthermore, Linser et al. (1977) have shown by electron microscopy (EM) that MVMp particles cluster around cellular filopodia immediately prior to endocytosis in mouse LA9 fibroblasts, indicating that the entry of MVMp into the cell could occur at the leading edge of migrating fibroblasts.

During cell migration, many cellular components are involved in the complex interactions that connect the extra-cellular matrix (ECM) to the cytoskeleton via trans-membrane receptors. For example, it is now well established that α5β1-integrins bind to the ECM protein fibronectin (FN) to form focal adhesion complexes at the leading edge of migrating cells (Burridge et al., 1997; Wu et al., 1993). This allows recruitment of numerous cellular proteins to the cytoplasmic tail of integrins (Burridge et al., 1997; Lawson et al., 2012; Sieg et al., 1999), and eventually to the activation of various signaling pathways by the small GTPases of the RAS superfamily. These include RhoA, for stress fiber formation (reviewed by Tojkander et al. (2012)), Rac, for lamellipodia/pseudopodia formation (reviewed by Petrie and Yamada (2012)), and CDC42, for filopodia formation (reviewed by Mattila and Lappalainen (2008)). All these GTPases can stimulate elongation of actin filaments via their downstream targets to allow protrusion and forward movement of the cell (reviewed by Le Clainche and Carlier (2008)).

One of the latest transformations to occur during tumorigenesis is a switch from a non-migrating epithelial to a highly migrating mesenchymal cellular phenotype (reviewed by Lamouille et al. (2014) and Savagner (2010)), a process named epithelial–mesenchymal transition (EMT). During EMT, the downregulation of the expression of the cell–cell junction protein E-cadherin (E-cad) and an increase in FN and N-cadherin (N-cad) expression facilitate the detachment and directed migration of individual cells. This is often a sign of poor prognosis, as it allows primary tumors to reach the blood vessels and spread throughout the organism, invading secondary sites, and leading to cancer metastasis (reviewed by Chaffer and Weinberg (2011) and Chiang and Massague (2008)).

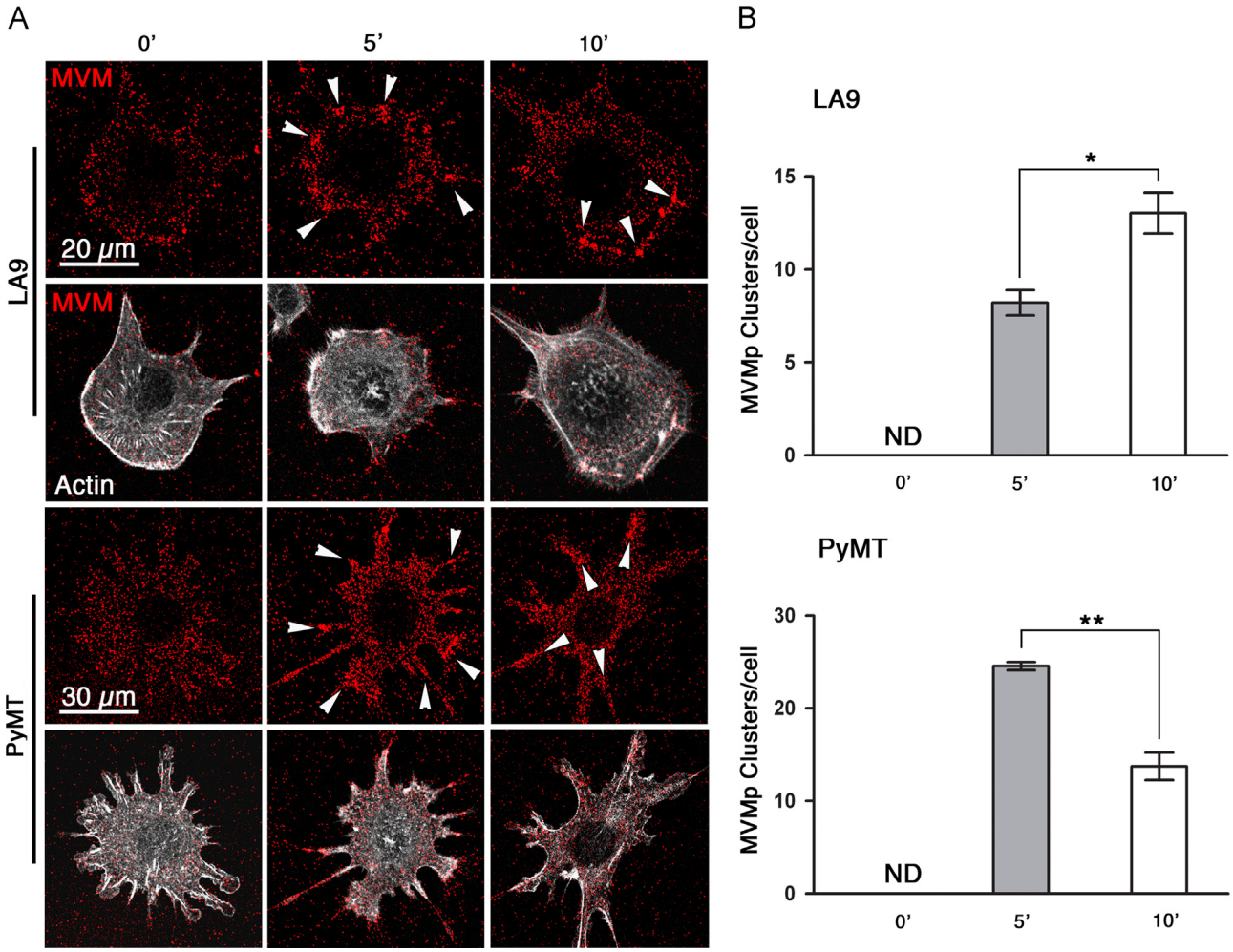

Fig. 1. Time course of MVMp clustering. (A) Cells were grown on glass coverslips, incubated with MVMp at a MOI of 8 for 2 h at 4 °C, shifted to 37 °C for 0, 5, or 10 min, and then prepared for IF microscopy. MVMp (red) was detected using a specific anti-capsid antibody. Actin filaments (pseudocolored in white) were labeled using AlexaFluor 647-conjugated phalloidin. Arrowheads point to MVMp clusters. (B) Quantification of the number of MVMp clusters (bigger than 50 or 30 pixels for LA9 and PyMT cells respectively) per cell for all the conditions from three independent experiments performed as described in A. Bar graphs show the mean ± standard error of the mean; $n=100$; $^{*}p<0.05$; $^{**}p<0.01$, as determined by unpaired Student t-test. 0′: 0 min; 5′: 5 min; 10′: 10 min. ND: not detectable.