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Imaging analysis of nuclear antiviral factors through direct detection of incoming adenovirus genome complexes

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ABSTRACT

Recent studies involving several viral systems have highlighted the importance of cellular intrinsic defense mechanisms through nuclear antiviral proteins that restrict viral propagation. These factors include among others components of PML nuclear bodies, the nuclear DNA sensor IFI16, and a potential restriction factor PHF13/SPOC1. For several nuclear replicating DNA viruses, it was shown that these factors sense and target viral genomes immediately upon nuclear import. In contrast to the anticipated view, we recently found that incoming adenoviral genomes are not targeted by PML nuclear bodies. Here we further explored cellular responses against adenoviral infection by focusing on specific conditions as well as additional nuclear antiviral factors. In line with our previous findings, we show that neither interferon treatment nor the use of specific isoforms of PML nuclear body components results in co-localization between incoming adenoviral genomes and the subnuclear domains. Furthermore, our imaging analyses indicated that neither IFI16 nor PHF13/SPOC1 are likely to target incoming adenoviral genomes. Thus our findings suggest that incoming adenoviral genomes may be able to escape from a large repertoire of nuclear antiviral mechanisms, providing a rationale for the efficient initiation of lytic replication cycle.

1. Introduction

Host cells have developed intrinsic mechanism(s) against invading viruses, which viruses try to evade on their part by evolving countermeasures against the cellular antiviral responses [1,2]. Cellular intrinsic defense mechanisms include the sensing and suppression of incoming viral genomes [2,3]. In this context, the interplay between cells and viruses has been best studied with herpes simplex virus type-1 (HSV-1) [3]. It has been shown that upon HSV-1 infection, incoming viral genomes are immediately targeted by PML nuclear bodies (PML-NBs) and/or its components [3]. Everett and co-workers have extensively studied the mechanisms of how PML-NBs respond against HSV-1 infection and demonstrated that ICP0, an E3 ubiquitin ligase encoded by the immediate-early gene of HSV-1, is a key factor to counteract the PML-NB-mediated repression [4]. It was shown that ICP0 induces degradation of PML, a central component of PML-NBs [5], and that the replication-defective phenotype of ICP0-null viruses can be rescued by depletion of PML, as well as of other PML-NB components [6–8]. Another important antiviral factor is IFI16, a nuclear DNA sensor that can induce the IRF3 and NF-κB signaling pathways [9]. Recent studies have revealed that IFI16 is immediately recruited onto incoming HSV-1 genomes for repression [10,11], but this process can be inhibited by ICP0 [10,12]. Intriguingly, it has been reported that IFI16 knockdown reduces the recruitment of PML-NB components onto HSV-1 genomes [10], suggesting a potential link between the two mechanisms.

In contrast to the non-chromatinized herpesviral genome, the adenovirus (Ad) genome is a linear double-stranded DNA forming a chromatin-like complex with viral basic core proteins inside the virion [13]. Core protein VII is the major, most abundant genome-bound factor, and the association with the viral genome has been thought to last at least during the first hours of infection even after nuclear import, as demonstrated by biochemical and microscopic analyses [14–17]. Ad genomes, like their herpesviral counterparts [2], are transported to the nuclear pore complex (NPC) still associated with the viral capsid, likely shielding it from

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cellular recognition [18]. At the NPC, the genome separates from the capsid and is imported into the nucleus where it can be targeted by cellular factors [18]. It has been shown that the host nuclear protein TAF-I/SET binds to Ad genomes through the interaction with protein VII upon nuclear import of the genomes [15,17]. Thus, protein VII and TAF-I can be used as surrogate markers to visualize the localization of incoming Ad genome complexes in cells [17]. Recently we have conducted detailed imaging analyses and reported that unlike HSV-1, incoming Ad genome complexes neither target nor are targeted by PML-NBs and/or its components up to 4 hpi (hours post infection) [19]. This suggests that cellular responses against nuclear replicating DNA viruses may differ between viral systems. However, as PML-NB formation and/or expression of its components is enhanced by interferon (IFN) treatments [20], IFN may augment antiviral properties of PML-NBs against incoming Ad genome complexes. In addition, given the reported isoform-specific roles of PML-NB components in virus control [21], it remains possible that a certain isoform(s) of the components might play a distinct role against (or in favor of) Ad infection. Indeed, it was reported that the PML-NB resident factor Sp100 is regulated in an isoform-specific manner during Ad infection, suggesting that Sp100A promotes viral transcription while the other isoforms Sp100B, C, and HMG seem to play an opposite role [22,23]. In contrast to PML-NBs, no study has yet directly analyzed if IFI16 targets incoming Ad genome complexes. The cellular chromatin protein PHF13/SPOC1 is another factor that has been described recently to possess potential antiviral functions against Ad infection [24]. It was proposed that PHF13/SPOC1 targets incoming Ad genome complexes for repression, but genome-associated protein VII may protect the viral genome from the PHF13/SPOC1-mediated antiviral response by binding to PHF13/SPOC1 [24]. However, formal evidence for the targeting of incoming Ad genomes by PHF13/SPOC1 is currently missing.

To extend our understanding of how the host cell responds against Ad infection, here we used our previously developed imaging approaches for the visualization of individual incoming Ad chromatin complexes in living cells and fixed materials [17] to examine how the different potential antiviral factors behave upon infection. In particular we tested IFN treatments and the use of specific isoforms of Sp100, but failed to observe co-localization between PML-NB components and incoming Ad genome complexes, as was observed in the previous study [19]. Furthermore, our imaging analyses indicated no specific changes in the localization of IFI16 and PHF13/SPOC1 upon Ad infection. Taken together, our findings suggest that incoming Ad genome complexes may be able to escape from and/or fail to be recognized by several nuclear antiviral factors/mechanisms, which target herpesviruses such as HSV-1.

2. Materials and methods

2.1. Cells and viruses

U2OS (ATCC #HTB-96) cells were maintained in DMEM Glutamax (Life Technologies) supplemented with 10% of fetal calf serum (FCS). Recombinant replication-competent human adenovirus type 5 (Ad5) and replication-deficient E1-deleted GFP-expressing Ad5 vector (Ad5-GFP) were amplified and purified as described previously [25,26]. The transfection of plasmids was done using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. For the interferon α (IFNα) treatment, cells were incubated in the presence of 1000 units/mL IFNα (PBL Assay Science) for 18 h before infection.

2.2. Antibodies

Antibodies used in this study are as follows: rat anti-protein VII [14], mouse anti-protein VII [17], mouse anti-IFI16 (Abcam, ab55328), and mouse anti-PML (sc-966, Santa Cruz Biotechnology), and rat monoclonal anti-PHF13/SPOC1 (6F6) [27] antibodies. Rabbit anti-Sp100 antibody was generously provided by T. Sternsdorf (Research Institute Children's Cancer Center Hamburg).

2.3. Plasmids

The expression vectors for EGFP-tagged and mCherry-tagged TAF-Iβ (pEGFP-C1-TAF-Iβ and pCHA-puro-FLAG-mCherry-TAF-Iβ) are described elsewhere [17,19]. The expression vectors for mCherry-tagged IFI16 and PHF13/SPOC1 (pcDNA3-IFI16-mCherry and pcDNA3-PHF13-mCherry) were obtained from MGC Montpellier Genomic Collections (Institut de Genetique Moleculaire de Montpellier). The expression vectors for EYFP-tagged Sp100A and Sp100B (pEYFP-C1-Sp100A and pEYFP-C3-Sp100B) were kindly provided by S. M. Janicki (Wistar Institute) [23].

2.4. Immunofluorescence and live-cell imaging analysis

Indirect immunofluorescence (IF) and live-cell imaging analyses were performed as described previously [17,19,28]. For pre-extractions, cells were first incubated with Transport buffer containing 0.5% Triton X-100 [17] and then fixed with 4% paraformaldehyde. IF samples were analyzed by a Leica SP5 confocal microscope. Confocal stacks were taken every 0.3 μm, and images were processed using ImageJ and presented as maximum intensity projections. For live-cell imaging, cells were seeded in ibidi μ-slide VI$^{0.4}$ (Ibidi), and images were acquired using a Leica spinning-disk microscopy system (x100 objective) equipped with an incubation chamber at 37 °C. Frames were taken every 3 s for each color channel and assembled into movies using MetaMorph software.

3. Results and discussion

3.1. IFN treatments or the use of specific Sp100 isoforms do not enable PML-NBs to target incoming Ad genome complexes

IFN is well known to enhance the expression of PML-NB components [20], possibly enforcing the antiviral role of the domains. This led us to test the possibility that IFN treatments may permit PML-NBs to target incoming Ad genome complexes. U2OS cells were first pre-treated with IFNα (or not) and then infected with Ad5, followed by IF analyses at 3 hpi using antibodies against PML and protein VII (Fig. 1A and B). As expected, the IFNα treatment enhanced the expression of PML as shown by an increased nuclear signal with anti-PML antibody (Fig. 1A). Increased expression of PML was also confirmed by Western blotting (Sup Fig. S1A). The results showed that irrespective of the IFNα treatment and despite the increase of PML expression, at 3 hpi no co-localization with protein VII foci was observed (Fig. 1B), consistent with a recent report using HDF-TERT cells [29]. This result suggests that PML-NBs do not target incoming Ad genomes even if stimulated through IFN treatments and/or upon increased expression of its components.

Next we tested if Sp100 isoforms exhibit distinct behaviors during immediate early phases of infection, as they were suggested to undergo isoform-specific regulations to regulate Ad gene expression [22]. First, we performed IF analyses using the specific antibody against Sp100 to investigate the localization of all isoforms of the endogenous protein at 3 hpi (Fig. 1C). Consistent with