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The DNA damage response to non-replicating adeno-associated virus: Centriole overduplication and mitotic catastrophe independent of the spindle checkpoint

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ABSTRACT

Adeno-associated virus (AAV) type 2 or UV-inactivated AAV (UV-AAV2) infection provokes a DNA damage response that leads to cell cycle arrest at the G2/M border. p53-deficient cells cannot sustain the G2 arrest, enter prolonged impaired mitosis, and die. Here, we studied how non-replicating AAV2 kills p53-deficient osteosarcoma cells. We found that the virus uncouples centriole duplication from the cell cycle, inducing centrosome overamplification that is dependent on Chk1, ATR and CDK kinases, and on G2 arrest. Interference with spindle checkpoint components Mad2 and BubR1 revealed unexpectedly that mitotic catastrophe occurs independently of spindle checkpoint function. We conclude that, in the p53-deficient cells, UV-AAV2 triggers mitotic catastrophe associated with a dramatic Chk1-dependent overduplication of centrioles and the consequent formation of multiple spindle poles in mitosis. As AAV2 acts through cellular damage response pathways, the results provide information on the role of Chk1 in mitotic catastrophe after DNA damage signaling in general.

Introduction

It is becoming increasingly clear that viruses often induce a DNA damage response in the infected cell (reviewed in Lilley et al. (2007)). Various triggers for this response have been suggested, including the incoming viral DNA, replication intermediates and virally encoded proteins, which may bind to DNA or themselves modify cellular DNA repair proteins (Lilley et al., 2007; Xie and Scully, 2007). In principle, a DNA damage response mounted against an infecting virus may be a cellular defense mechanism that the virus must circumvent or override, or it may be a cellular reaction that the virus can exploit for its own replication. The two are not mutually exclusive.

In the case of parvoviruses the ability to induce a DNA damage response and to perturb cell cycle progression has been established. Adeno-associated virus (AAV2) (Raj et al., 2001; Winocour et al., 1988), the B19 virus (Morita et al., 2003, 2001) and the minute virus of mouse (MVM) (Anouja et al., 1997; Op De Beeck et al., 1995) can all provoke a cell cycle arrest. AAV2 induces a cell cycle block in the G2 or G1 phase of the cell cycle (Fragkos et al., 2009; Raj et al., 2001; Winocour et al., 1988), and B19 virus can provoke an arrest in either G2 (Morita et al., 2001) or in the G1 phase through the action of the non-structural regulatory protein NS1 (Morita et al., 2003). MVM triggers an arrest in G2 that is mediated by the viral NS1 protein (Anouja et al., 1997; Op De Beeck et al., 1995). With AAV2, the virus-induced DNA damage response and cell cycle arrest can be mediated either by certain of the AAV2 Rep proteins (Berthet et al., 2005; Saudan et al., 2000; Schmidt et al., 2000), or by a mechanism based on the DNA structure; in this latter case the response can be triggered as well by both wild type and UV-inactivated virus (Fragkos et al., 2008; Raj et al., 2001; Winocour et al., 1988). In cells infected with AAV2 or UV-inactivated AAV2 at moderate to high multiplicites, DNA repair proteins co-localize with the viral genomes in virus-induced nuclear foci (Jurvansuu et al., 2005). The consequences of the AAV2-induced DNA damage signaling are activation of p53 (Raj et al., 2001) and of the checkpoint kinases ATR and Chk1 (Jurvansuu et al., 2005), which leads to cell cycle arrest (Jurvansuu et al., 2005; Raj et al., 2001). Of particular interest is that this response to AAV2 can cause cell death in p53-deficient cells (Raj et al., 2001). It was recently shown that cells with a defective p53–p21–pRb pathway are susceptible to AAV-induced killing (Garner et al., 2007). In AAV2-infected cells (Jurvansuu et al., 2007) or following genotoxic stress (Zhang et al., 2005), Chk1 is unstable. Thus, AAV2-infected p53-deficient cells cannot sustain the prolonged arrest in G2 and enter mitosis in the face of damage signaling (Jurvansuu et al., 2007), whereas cells with an intact p53 pathway remain arrested. Thus, p53 in this situation, rather than inducing cell death, unexpectedly protects cells against it.

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The cellular response to DNA damage by radiation or drugs, and the ensuing modes of cell death, have been extensively studied (reviewed in Blank and Shiloh (2007)). Various models of cell death by apoptosis, or death during or after mitosis, have been reported, sometimes even within the same population of cells (Dodson et al., 2007; Eom et al., 2005). But what determines how and when the cells die remains unclear. The activity of the mitotic spindle checkpoint is required for mitotic catastrophe and cell death induced by DNA damaging agents (Cheung et al., 2005; Fang et al., 2006; Nitta et al., 2004; Vogel et al., 2007, 2005). In the case of drugs targeting microtubules or microtubule motor proteins, the role of the spindle checkpoint in cell death remains controversial (Chin and Herbst, 2006; Dowling et al., 2005; Gascoigne and Taylor, 2008; Kienitz et al., 2005; Lee et al., 2004; Masuda et al., 2003; Sihn et al., 2003; Sudo et al., 2004; Tao et al., 2007, 2005; Taylor and McKeon, 1997; Vitale et al., 2007). Centrosome overduplication and the resulting spindle pole abnormalities have been described as playing an important role in mitotic catastrophe following DNA damage (Dodson et al., 2007; Kawamura et al., 2004; Sato et al., 2000a,b). Chk1 activation and centrosomal localization may have a role in such centriole overduplication (Bourke et al., 2007; Katsura et al., 2009; Loffler et al., 2007; Robinson et al., 2007), but it is not clear if this is direct or not.

We have used AAV2 and UV-inactivated AAV2 to trigger a DNA damage response that leads to death by mitotic catastrophe of p53-defective cells (Jurvansuu et al., 2007, 2005; Raj et al., 2001). Chk1 was identified as a critical determinant, firstly of cell cycle arrest and subsequently of passage into M phase (Jurvansuu et al., 2007). Thus there is a seeming paradox in that UV-AAV2 infection causes cell death in mitosis although the infection does not provoke detectable breakage of cellular DNA (Fragkos et al., 2009; Jurvansuu et al., 2007). In the work reported here we used U2OS osteosarcoma and HCT116 colon cancer cells to show that, in the p53-deficient cells, the virus triggers a striking Chk1-dependent overduplication of centrioles that leads to the formation of multiple spindle poles in mitosis and consequent mitotic catastrophe. AAV2 acts through cellular DNA damage response pathways, therefore the results also provide information about the role of Chk1 and the spindle checkpoint in mitotic catastrophe after DNA damage signaling in general.

Results

UV-AAV2-infected U2OS + p53DD cells enter mitosis after a prolonged arrest in G2 but are unable to divide and die as a consequence of impaired mitosis

It was shown previously that wild type or UV-inactivated AAV2 (UV-AAV2) provoke a DNA damage response in infected cells that leads to an arrest at the G2/M border of the cell cycle (Raj et al., 2001). In the absence of an intact p53 pathway, this arrest cannot be sustained leading to cell death. To investigate the stage at which UV-AAV2-infected p53-negative cells die, we stably transfected U2OS + p53DD cells with a green fluorescent protein (GFP)-tagged histone H2B in order to follow the dynamics of the infected cells. Cells were filmed during a 24 h period and every 10 min a bright field and a fluorescent image were captured. Time-lapse microscopy revealed that uninfected U2OS cells round up and go through mitosis with an average prometaphase-to-telophase transition time of 90 min (Fig. 1A). In contrast, UV-AAV2-infected cells round up and enter mitosis three days post-infection but are unable to divide and arrest in mitosis (Fig. 1A). The outcome of the UV-AAV2 DNA damage signaling-induced mitotic arrest was cell death, accompanied by DNA fragmentation (Fig. 1A, time 540 min). The duration of the mitotic arrest was 8.6 h on average for the UV-AAV2-infected cells compared to a time in mitosis of 1.5 h for uninfected control cells (Fig. 1B).

For quantification, cells were classified as dying either of defects in mitosis (mitotic catastrophe) or of apoptosis independently of mitosis. The cells that died of mitotic defects included cells that condensed their chromatin into prometaphase structures and then tried unsuccessfully to divide, giving the appearance of being stuck in mitosis, as well as cells that on trying to divide formed micronuclei and finally died. The apoptotic cells, on the other hand, did not enter mitosis but displayed apoptotic features without showing the prometaphase-like chromatin pattern. This form of cell death was, unlike mitotic death, usually very rapid. On filming cells during 21 h four days after infection, the time-lapse recordings revealed that 83% of the UV-AAV2-infected cells that were in the process of dying did so in mitosis while 17% of the dying cells did not enter mitosis and were characterized as apoptotic (Fig. 1C). Cells that had formed micronuclei during the first cell division were unable to go through a second round of cell division and died in G1 (data not shown). These results led us to conclude that the majority of UV-AAV2-infected cells die as a consequence of a failure of mitosis; nevertheless a proportion of cells die through apoptosis without entry into mitosis.

The infected mitotic cells display an increased number of spindle poles containing centrioles

To examine the UV-AAV2-infected mitotic cells in more detail we used immunofluorescent microscopy to visualize the microtubule network with an antibody against alpha-tubulin and co-stained the centrioles with centrin-3, a centriolar marker. DAPI was used to stain the nucleus. The majority of uninfected U2OS cells displayed a bi-polar spindle structure in mitosis (Fig. 2A). Interestingly, in the UV-AAV2-infected cells four days after infection, we could observe a large increase in the percentage of cells with multiple spindle poles compared with uninfected cells. Moreover, as shown in Fig. 2A, centrin-3 staining revealed that the multiple spindle poles contained centrioles. The multipolar phenotype was also observed in p53-negative HCT116 cells (HCT116p53−/−). HCT116p53−/− cells were stained with an antibody against α/β tubulin and the DNA was counterstained with DAPI in order to quantify the number of mitotic cells with multipolar spindles. Four days post-infection we observed that 58.4% of the cells displayed multipolar spindles compared with 5.3% in the uninfected cells (Fig. 2B). In this and subsequent experiments, the Student's paired *t*-test, two-tailed distribution was used. These results show that UV-AAV2-induced DNA damage signaling leads to amplified centriole numbers and the formation of multipolar mitotic spindles in p53-negative cells.

Interphase cells infected with UV-AAV2 display amplified centrosome numbers

To further examine the centrosome numbers in the UV-AAV2-infected cells we stained U2OS + p53DD cells with the centrosomal marker γ-tubulin and the DNA with DAPI. The γ-tubulin staining showed that the majority of the uninfected cells contained 1-2 centrosomes depending on the cell cycle stage, whereas in the UV-AAV2-infected cells we could see a high increase in centrosome numbers in interphase cells (Fig. 3A). The centrosome numbers varied from 3 to a maximum of 21 (data not shown). To determine if centrosomes of interphase cells contained centrioles we stained U2OS + p53DD cells with the centriolar marker C-Nap1 (Fig. 3B), which stains the proximal ends of mother centrioles (Fry et al., 1998). We also quantified the number of cells with more than two C-Nap1 foci in both U2OS and U2OS + p53DD cells, which gave similar results (Fig. 3C). We found that two days post-infection 72% of the UV-AAV2-infected U2OS cells displayed more than two C-Nap 1 foci compared with 4% in the uninfected control cells (Fig. 3C). In U2OS + p53DD cells we found that 71.6% of the UV-AAV2-infected cells two days