Adenovirus Release from the Infected Cell as a Key Factor for Adenovirus Oncolysis

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Abstract: Adenovirus release is not triggered until late times after viral infection, when the adenovirus death protein (ADP) accumulates to induce viral egress. Thus, the natural rate of adenovirus release may hinder the spread of oncolytic adenoviruses. Several experimental approaches have provided evidence indicating that promoting adenovirus release can be used to enhance their therapeutic potential. This review briefly summarizes what is known about the mechanism of adenovirus release and describes three different strategies, ADP overexpression, apoptosis induction, and bioselection, which can be used to enhance adenovirus release. Finally we will discuss some of the future perspectives that will contribute to the better use of progeny release for the improvement of the antitumor activity of oncolytic adenoviruses.

Keywords: Adenovirus, progeny release, cell-to-cell spread, oncolytic potency.

INTRODUCTION

Viral spread and pathogenesis is determined by the efficiency of viral exit from the infected cell. The release of adenovirus is rather inefficient since it occurs long after infection when the adenovirus death protein (ADP) has accumulated to promote viral egress. Only 20% of the adenovirus produced is released by 48 h post-infection. This has led to the idea that the natural rate of adenovirus release hinders the intratumoral spread of oncolytic adenoviruses. The large plaque phenotype indicative of improved cell-to-cell spread yielded with specific genetic modifications of adenovirus was the first evidence that augmenting progeny release could be used to enhance the spread of oncolytic adenoviruses. Overexpression of ADP [1] or functional deletion of E1b-19kD [2] were able to enhance the cytotoxicity of adenovirus by inducing early cell lysis and enhancing progeny release. Moreover, Wein and collaborators used a mathematical model to study the complex interactions between tumor cell growth, virus replication, and the immune system. They concluded that viral replication would only eliminate the tumor when the rate of cell lysis and progeny release outpaced tumor cell growth [3]. In contrast, another study by Wodarz suggested that maximizing the death rate of infected tumor cells could decrease viral production and result in suboptimal outcome of treatment [4]. Supporting Wein's hypothesis, several reports have demonstrated that genetic modifications which improve adenovirus release enhance the antitumor activity of oncolytic adenoviruses in tumor xenografts [5, 6]. In addition, both *in vitro* and *in vivo* bioselection of mutants with improved spread or oncolytic potency led to release-enhancing mutations, highlighting the importance of adenovirus release for oncolysis [7-9]. According to this

evidence, the rate of progeny release is a key parameter controlling the spread and antitumoral efficacy of oncolytic adenoviruses. This review outlines the biology of adenovirus cell death and progeny release, summarizes the strategies which can be used to enhance the release of oncolytic adenoviruses, and highlights several considerations to choose a release enhancing modification.

BIOLOGY OF ADENOVIRUS CELL DEATH AND PROGENY RELEASE

To date, the mechanism of adenovirus cell lysis and progeny release remains poorly understood. Several different groups have attempted to characterize adenovirus-induced cell death at late stages of infection, but the exact mechanism remains controversial. Zhang and Schneider first demonstrated that host cell shutoff of protein synthesis was necessary to facilitate cell lysis and release of infectious progeny virus particles [10]. Treatment of adenovirus-infected cells with 2-aminopurine prevented host-cell shutoff and yielded a 200-fold reduction of progeny release without significantly affecting viral production. However, the contribution of this process to adenovirus release remains to be elucidated. Subsequent studies revealed that adenovirus encodes for several proteins which both inhibit or promote cell death and act in concert to control the fate of the infected cell and the exit of the viral progeny. E1B-19kD together with E1B-55kD and E4orf6 are expressed early after infection and act as antiapoptotic proteins preventing premature apoptotic cell death induced by E1A [11-13]. Deletion or loss of function of E1b-19kD yields a large plaque phenotype, indicative of improved cell-to-cell spread due to enhanced cell killing and progeny release, but has also been reported to decrease viral production [8, 14]. Therefore, the importance of this protein relies on its ability to delay cell death to ensure efficient viral replication and generation of new virions.

At late times after adenovirus infection, virions accumulate in the nucleus, intermediate filaments are disrupted,

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the cell rounds-up evidencing the typical cytophathic effect (CPE), and cell membrane permeabilization allows virions to escape into the extracellular medium. The E3-11.6K protein, also known as adenovirus death protein (ADP), has a major role in this process. Deletion of this protein results in an impaired release without affecting viral production, and cells infected with these mutants have small, slow developing plaques. Although the exact function of this protein is still unknown, the accumulation of this protein late in the adenovirus cycle is required for efficient cell lysis and release of viral particles into the extracellular medium [15, 16]. Interestingly, although E3-11.6K is encoded within the early E3 transcriptional unit it is synthesized only in small amounts from the E3 promoter, but very abundantly from the major late promoter starting at about 20 to 25 h post-infection [17]. Additionally to ADP, E4orf4 has also been found to induce cell death [18] and has been suggested to collaborate with ADP inducing cell lysis and progeny release. In fact, although less evident than ADP-deleted viruses, E4orf4-null viruses exhibit a prolonged delay of cell death [19], supporting a role for E4orf4 in this process.

More recently, a work from Abou el Hassan *et al*. indicated that the cell killing induced by an oncolytic adenovirus was independent from the basic apoptotic machinery and termed the oncolysis induced by the virus as 'necrosis-like' [20]. In a later study, Ito *et al*. coupled, for the first time, adenovirus induced cell death with autophagy. They found that infection of glioma cells with a replicative adenovirus with E1A expression controlled under the hTERT promoter (hTERT-Ad) induced acidic vesicular organelle development and increased the localization of LC3 to the autophagosome membrane (enhanced LC3-II to LC3-I ratio), two typical features of autophagy [21]. Supporting this type of cell death, Jiang *et al*. also described AdΔ24RGD induced autophagic cell death in glioma stem cells, as indicated by accumulation of Atg5 and LC3-II protein and autophagic vacuoles [22]. Furthermore, enhancement of autophagy in glioma cells through combination with RAD001 improved the antitumoral effect of oncolytic adenovirus AdΔ24RGD *in vitro* and *in vivo* [23]. Contrary to these results, Baird *et al*. found that 3-methyladenine and chloroquine, two drugs which inhibit early autophagic signaling and autophagosome and lysosome fusion, respectively, augmented virus-induced death in ovarian cancer cell lines in a dose dependent manner [24]. A more recent work by Ulasov *et al*. carefully studied apoptosis and autophagy involvement in adenovirus induced oncolysis in glioma cells. Their results, which include both non-classical apoptosis and autophagy features, indicated that apoptosis may not be a terminal stage of virus-induced toxicity and that autophagy could explain some of the proapoptotic features observed [25]. All together, these results discard classical apoptosis and provide evidence for autophagy induction at late stages of adenovirus infection. However, further experiments will be required to demonstrate whether autophagy contributes to adenovirus induced cell death or acts as a cell survival response to adenovirus infection.

METHODS TO INCREASE ADENOVIRAL SPREAD

Several encouraging strategies are being explored to generate oncolytic adenoviruses with enhanced release and improved potency. In this review, the various viruses will be organized into three main categories, according to the strategy that has been used to enhance their release. The first category includes oncolytic adenoviruses which overexpress the E3 protein ADP. The second category includes strategies to induce apoptosis in the infected cell. Finally, the third category includes those mutants obtained through bioselection of randomly mutagenized pools of adenoviruses. Table **1** summarizes current replication-competent adenoviruses with enhanced release, and their main characteristics.

ADP Overexpression

After the identification of ADP as a key element for efficient cell lysis and release of virions from infected cells, a number of adenoviruses that overexpress ADP have been constructed with the aim of improving their oncolytic potential. In these viruses, ADP overexpression has been achieved by using two main strategies.

The first strategy consists of shifting the expression of ADP from late to early to accumulate larger amounts of ADP at earlier stages and make cell lysis and release more efficient. To accomplish this, Doronin *et al*. deleted all the E3 genes except ADP or all the E3 genes except E3-12.5K and ADP, to generate KD1 and KD3, respectively [1]. The deletion of these genes changes the fine transcriptional balance of the E3 transcriptional unit favoring the transcript that encodes ADP leading to its overexpression. KD1 and KD3 also have two E1A mutations that preclude binding of E1A proteins to p300 and pRB, and render these viruses selective for dividing cancer cells. Using these viruses, they demonstrated for the first time that ADP overexpression produces early cytophatic effect and increases cell-to-cell spread without impairing viral production due to a premature cell lysis. Importantly, both KD1 and KD3 were more effective at suppressing subcutaneous lung cancer tumors *in vivo* than a control virus, which expressed wild-type levels of ADP. Later, this strategy has been used by the same authors for the construction of two replication competent adenoviruses expressing a wild-type E1A protein, VRX-006 (lacking all the E3 genes except 11.6K) and VRX-007 (lacking all the E3 genes but 12.5K and 11.6K) and several other oncolytic adenoviruses such as VRX-009 and VRX-011 (based on VRX-007) or KD1-SPB (based on KD1). In each case, it was found that ADP overexpression produced early cytophatic effect, early cell lysis, large plaque, and increased cell-to-cell spread resulting in an improved therapeutic potential [5, 6, 26, 27].

The second strategy involves the replacement of the entire E3 coding region with an expression cassette containing the *ADP* gene controlled by a promoter. Using this approach, Ramachandra *et al*. constructed the oncolytic adenovirus 01/PEME, in which an extra copy of the viral MLP was placed into the E3 region for overexpression of ADP [28]. 01/PEME displayed increased CPE in cell culture and enhanced antitumor activity *in vivo* compared to viruses that do not overexpress ADP. In another study, ADP was expressed under the control of the CMV promoter (YKL-cADP) [29]. ADP expression from the CMV promoter produced earlier cell lysis and enhanced viral release compared to ADP expression from the extra MLP (YKL-

Table 1. Examples of Replication-Competent Adenoviruses with Enhanced Progeny Release

Viruses	Genetic Modifications to Increase Release	ADP	E3 Genes Deleted	Selective Replication	Reference
ADP overexpression					
KD1 KD3	deletion of E3 genes	overexpressed	all but 11.6K all but 12.5 and 11.6K	YES	[1]
VRX-006 VRX-007	deletion of E3 genes	overexpressed	all but 11.6K all but 12.5K and 11.6K	NO	[27]
01/PEME	Insertion of a cassette containing *adp* gene and MLP	overexpressed	all but 12.5K	YES	[28]
YKL-cADP YKL-mADP	Insertion of a cassette containing *adp* gene and: CMV promoter MLP	overexpressed	all but 12.5K	YES	[29]
Ad5-yCD/ mutTKSR39-rep-ADP	Insertion of a cassette containing *adp* gene and: CMV promoter	overexpressed	all	YES	[30-31]
dl716	C-terminal truncation of ADP protein	overexpressed	none	NO	[34, 36]
dl732	Mutation of the splicing acceptor near *adp* coding sequence	overexpressed	none	NO	[35, 36]
Apoptosis induction					
Ad337	E1b-19kD deletion	wild-type	E3B (14.7K, 14.5K, 10.4K)	YES	[2, 37]
dl250	E1b-19kD deletion	wild-type	none	YES	[38, 39]
Adp53rc	Insertion of p53 gene after the fiber gene	deleted	all	NO	[50]
AdΔ24-p53	Insertion of p53 expression cassette in the deleted E3 region	deleted	all	YES	[51-53]
Ad5/35.1R-E1A/TRAIL	Insertion of E1A-TRAIL expression cassette replacing the deleted E1 region	wild-type	none	YES	[57, 58]
ZD55-TRAIL	Insertion of CMV-TRAIL cassette replacing the deleted E1b-55K gene	wild-type	none	YES	[59, 60]
Bioselection of mutants					
Lp53	C-terminal truncation of i-leader protein	deleted	all but 12.5K	NO	[8]
Lp11 Lp26	Defective in E1b-19K Defective in E1b-19K and E1b-55K	deleted	all but 12.5K	NO	[8]
ONYX-201 ONYX-203	C-terminal truncation of i-leader protein	wild-type	none	NO	[9]
AdT1	C-terminal truncation of E3-19kD protein	wild-type	none	NO	[7]

mADP). Moreover, the enhanced potency of YKL-cADP adenovirus over YKL-mADP also translated into a greater antitumor efficacy and survival advantage *in vivo* in both a cervical and hepatoma xenograft tumor models. Finally, ADP expression under the control of the CMV promoter has also been used by another group to generate a second-generation armed adenovirus, Ad5-yCD/mutTKSR39rep-ADP [30]. Importantly, the safety of this virus has been established in a clinical trial [31], and now its efficacy is being evaluated in a phase III randomized clinical trial.

It is important to note that all the strategies described above to overexpress ADP require partial or total deletion of the E3 region (Table **1**). Although the loss of function of these proteins can be underestimated *in vitro*, several studies provide clear evidence demonstrating that deletion of some of these proteins favor the clearance of the virus in immunocompetent models [32, 33]. As an alternative to the previously mentioned viruses, *dl*716 and *dl*732 mutants overexpress ADP while preserving the E3 immuno-modulatory functions. These two mutants were used in early virologic works to study the E3 complex transcription unit as a model to understand alternative pre-mRNA processing [34, 35]. However, it was not until 2003 that these viruses were described to display large-plaque phenotype [36]. The *dl*732 mutant contains a 60 bp deletion which affects a splicing acceptor near the ADP coding sequence and results in earlier transcript synthesis and overexpression of this gene. The *dl*716 mutant has a 22 bp deletion that induces a c-terminal truncation of the ADP protein. Although to date these genetic modifications have not been applied to enhance the release of an oncolytic adenovirus, the preservation of the E3 immunomodulatory functions could be beneficial in presence

of an immune system, and therefore they deserve further investigation.

Apoptosis Induction

The first evidence that apoptosis could enhance progeny release of replicating adenoviruses was the large plaque phenotype displayed by an E1b-19K deleted adenovirus (dl337). Failure of this virus to inhibit E1A induced apoptosis during adenovirus infection led to premature cell death and enhanced release and spread [2]. Moreover, the dl337 mutant greatly enhanced the apoptotic effect of conventional chemotherapies, such as cisplatin and paclitaxel [2] *in vitro*, and was also better than wild-type adenovirus at reducing tumor growth in A549 tumor xenografts [37]. Soon after these earlier studies, another group further studied the selectivity and toxicity of adenoviral mutants lacking the E1b-19K gene with and without E3B gene expression. Overall, the adenoviral mutant with E1b-19K deletion and E3B retention (dl250) displayed enhanced viral spread and increased antitumoral potency compared to wild-type adenovirus in a variety of *in vitro* and *in vivo* tumor models [38, 39]. In addition, this mutant showed tumor necrosis factor-enhanced cancer selectivity due to genetic blocks in apoptosis pathways in cancer cells [38, 39]. Importantly, in all these studies, E1b-19K deletion did not impair viral replication, and both dl337 and dl250 grew to wild type levels, and sometimes even more. However, it is important to note, that in earlier virologic studies, E1b-19K mutants were found to be defective in non-complementing cell lines [14].

Another work presented by Mi *et al.* also supports the finding that apoptosis induction can result in earlier release of virions from infected cells. In this work, they used a system in which expression of a mutated form of I-kB (that avoids NF-kB activation) by an adenoviral vector (Ad.ikB$_M$) sensitized tumor cells to recombinant human TNF-α-mediated apoptosis [40]. Although the repeated intratumoral administration of TNF-α in mice bearing tumors of HeLa cells infected with Ad.ikB$_M$ had antitumor effect, this approach is not applicable in clinical settings because of potential side effects from systemically applied TNF and expression of I-kB$_M$ in non tumor tissues. However, this system served as a model to demonstrate that apoptosis induced after virion assembly facilitates virion release and enhances intratumoral spread. By contrast, apoptosis induced during viral DNA replication compromised virus production, indicating that careful timing of cell lysis is crucial. Importantly, this work demonstrated that apoptotic cell death leads to the engulfment of progeny virions into apoptotic bodies which can spread from cell to cell through phagocytosis of these apoptotic bodies. This cell death mode may be very valuable given the resistance of this type of spread to the presence of neutralizing antibodies.

A different strategy to induce apoptosis in infected cells is to arm viruses with exogenous transgenes that promote apoptosis. The p53 tumor suppressor protein is an attractive candidate that has been used for this purpose. p53 gene transfer has been shown to induce growth inhibition and apoptosis in a variety of cancer types [41-44]. By contrast, p53 gene transfer is relatively nontoxic to normal cells [45, 46]. Moreover, it has been suggested that functional p53 can promote the adenovirus lytic cycle [47-49]. On the basis of

these observations, two independent groups exploited the use of p53 with the aim of improving oncolytic potency. Sauthoff *et al.* demonstrated that late expression of p53 from a replicating E3-deleted virus improves tumor cell killing and viral spread without impairing viral replication [50]. Interestingly, p53 was more cytotoxic than ADP in cancer cells, but less cytotoxic than ADP in normal fibroblasts, improving therefore the specificity of tumor cell killing. p53 gene has also been inserted in place of the deleted E3 region in AdΔ24, to create AdΔ24-p53 [51]. AdΔ24-p53 exhibited enhanced oncolytic potency compared with AdΔ24 in most cancer cell lines tested independent of their p53 status. Moreover, AdΔ24-p53 was more efficient at suppressing tumor growth when compared to AdΔ24 in glioma [52] and neuroblastoma [53] xenograft models.

Another transgene that has been used to increase adenovirus spread is the TNF-related apoptosis-inducing ligand (TRAIL). TRAIL is a transmembrane protein that is processed into a soluble molecule able to kill many types of tumor cells *via* an apoptotic cascade [54], while not causing any toxicity *in vivo* [55, 56]. In the earliest study, Sova *et al.* constructed a tumor-targeted oncolytic adenovirus which expressed TRAIL in a tumor-selective manner. This virus did not contain the antiapoptotic E1b-55kD and 19kD genes, which can potentially inhibit TRAIL-mediated apoptosis. TRAIL expression by this virus improved virus release and cytotoxicity in a variety of human tumor cell lines *in vitro* [57, 58]. *In vivo*, the TRAIL-expressing virus showed an enhanced antitumor activity compared to its parental virus in a variety of tumor models, including intravenous administration in a liver metastasis model [57] and intratumoral administration in s.c. glioblastoma xenografts [58]. The ability of TRAIL to enhance viral release has been confirmed by another group that created an oncolytic adenovirus that expressed TRAIL (ZD55-TRAIL) by replacing the E1b-55kD gene with a CMV-TRAIL cassette [59]. Interestingly, ZD55-TRAIL has been used in combination with chemotherapeutic agents, such as 5-FU [59] and cisplatin [60], resulting in a greater antitumor activity than either treatment alone.

Bioselection

Another approach to generate viruses with enhanced release involves bioselection of randomly mutagenized adenovirus by repeated passaging under carefully controlled conditions. This genetic selection yields point mutations that are compatible with viral replication and has the potential to assign novel functions to viral genes. In the earliest work, Subramanian *et al.* used a bioselection strategy to identify Ad5 genes that play a role in viral spread [8]. In this study, an Ad5 mutant (dl327 lacking most of the E3 region) with small-plaque phenotype was randomly mutagenized by exposure to UV, and 27 mutants that were able to revert the reduced spread of the dl327 were selected. 23 out of 27 mutants contained mutations affecting the function of the E1B-19kD protein, which led the authors to hypothesize an opposing effect of E1B-19kD and ADP in adenovirus spread. Although E1b-19kD mutants formed large plaques in the absence of ADP, the presence of the E3 region in an E1b-19kD mutant did not significantly enhance plaque size or cytolysis, suggesting that ADP may antagonize the function

of E1B-19kD. Among the other compensatory mutants that were isolated, lp53 showed the most pronounced effect. This virus contains a single base pair change in the adenoviral genome resulting in a C-terminal truncation of the i-leader protein. The lp53 mutation showed additive effect with the E3 region and with a deletion of the E1B-19kD region. In contrast, the i-leader null mutation did not appear to influence viral spread. Therefore, the authors suggested that the full-length i-leader protein may be in an inactive conformation and may be functionally activated by proteolysis of the C-terminal region under specific conditions. Interestingly, a previous genetic selection of Ad5 mutants with enhanced oncolytic activity identified two mutants (ONYX-201 and ONYX-203) that also contained a C-terminal truncation in the i-leader protein [9]. This mutation appeared to be essential for the increased cytotoxic activity of these mutants, confirming the relevance of such mutations. Although the exact role of the i-leader protein in adenovirus replication is not clear, the fact that this protein encoded by the major late transcription unit is expressed prior to viral DNA replication led the authors to postulate that early accumulation of the truncated i-leader protein could accelerate viral DNA replication through an unknown mechanism.

Finally, in an effort to select a mutant with enhanced antitumor activity, our group extended the bioselection approach by performing repeated passages of a pool of Ad5 random mutants in an *in vivo* murine model of human cancer [7]. By using this approach, we isolated AdT1, a new mutant with improved viral release and enhanced systemic antitumor activity *in vivo*. A truncating mutation in the endoplasmic reticulum retention domain of the E3-19K protein (445A), which relocates the protein to the plasma membrane, was found to be the responsible for the enhanced release phenotype. How the relocalization of E3-19K improves adenovirus release is not clear to date, however, our results suggest that the E3-19K(445A) mutation is able to disrupt intracellular Ca^{2+} homeostasis and create membrane lesions that allow enhanced virus release. Further studies to elucidate the role of the new identified mutations in viral release, as well as the incorporation of these mutations in oncolytic adenoviruses become of increasing relevance.

FUTURE DIRECTIONS

Three main considerations must be taken into account when choosing a release enhancing modification: (a) modifications should not compromise virus replication, (b) point mutations or deletions rather than insertion of transgenes should be preferentially used to save space, and (c) deletion of E3 immunomodulatory proteins may affect the ability of the virus to evade the immune response and, therefore, should be minimized.

The importance of the immune response to oncolytic adenoviruses is paramount as the outcome of the therapy will depend, in great measure, on the interaction of the virus with the immune system [61]. Besides the implications of the deletions of E3 immunomodulatory proteins to enhance progeny release, it is possible that the improvement of adenovirus release, itself, could affect the immune response to an oncolytic adenovirus. It is generally believed that a prolonged infectious cycle (inefficient viral release) in the

case of a highly immunogenic virus such as adenovirus would allow the immune system to rapidly neutralize the infection without allowing virus to spread [62]. The faster the virus is released from the infected cell, the less chance the virus has of being killed in the cell by cytotoxic T lymphocytes, NK cells, and phagocytic cells and the greater chance it has to infect another cell [15]. Contrary to this hypothesis, the presence of more extracellular virus could possibly trigger a stronger innate response and, in turn, attract a more powerful adaptative antiviral response leading to a faster clearance of the virus. Despite this, the enhancement of adenovirus release has been reported to improve the spread and antitumoral potency of oncolytic adenoviruses in nude mice bearing human tumor xenografts models. However, the benefits of oncolytic adenoviruses with enhanced release have never been tested properly in presence of an immune system and the consequences of an improved release on the immunogenicity of an oncolytic adenovirus remain unexplored. So far, only two viruses with release enhancing modifications have been studied in Syrian hamsters, a model that is semipermissive to adenovirus replication: VRX-007 (all E3 gens deleted except 12.5K and ADP)[63] and AdT1 (Ad5 with E3/19K-445A mutation, which relocalizes E3-19K to the plasma membrane) [7]. In both cases, these viruses demonstrated the same antitumor efficacy as their wild-type counterparts. However, the release enhancing modifications of these viruses may also compromise other immune evading functions of adenovirus, making conclusions hard to extract. A more detailed study of the impact of release enhancing genetic modifications on the immune response to oncolytic adenoviruses would help predict if viruses with improved progeny release require combination with immune suppression when the objective is purely *virocentric*, or whether they could boost the immune response against the tumor when the final goal is *immunocentric* [61].

In addition to the immune response, tumor stroma may also play a prominent role in inhibiting viral spread and tumor cell killing of oncolytic adenoviruses. Solid tumors are characterized by small groups of tumor cells surrounded by large areas of tumor-associated fibroblasts and extracellular matrix (ECM). In addition, unfavorable pressure gradients within established tumors also inhibit diffusion of large molecules. Degradation of ECM with proteolytic enzymes, such as relaxin or hyaluronidase, has been shown to reduce interstitial fluid pressure, enhance adenoviral spread and improve viral potency *in vivo* [64-66]. Therefore, combining modifications to increase adenovirus release with strategies to disrupt intratumoral barriers may be important to maximize spread efficiency. Further studies on the use of promoters which allow the replication of oncolytic adenoviruses in tumor-associated fibroblasts as well as in tumor cells together with strategies to improve the spread within this cell type would also be of interest, as this cell population has been considered a major barrier for efficient virus spread.

Finally, a better understanding of the mechanism of viral release and the type of cell death mode triggered should promote the development of novel vectors with improved progeny release and contribute to enhance the antitumoral activity of oncolytic adenoviruses. In this sense, the study of

progeny release induced by other naked viruses could also produce new hypotheses. The process leading to viral egress of poliovirus or coxsackievirus, for example, has been extensively studied. Both viruses encode for small highly hydrophobic proteins, capable of modifying the infected cells permeability to ions or small molecules, also known as viroporins [67-69]. A protein with the ability to modify permeability has not yet been described for adenovirus, although several hypotheses suggest that the adenovirus death protein (ADP) could induce cell lysis and release through a similar mechanism [27]. Interestingly, early studies from 1970 demonstrated that adenovirus plaque formation can be enhanced in the presence of magnesium [70], suggesting that certain ions can improve the release and spread of adenovirus. In addition, our group isolated a mutant through an *in vivo* bioselection strategy capable of enhancing adenovirus release through alteration of calcium homeostasis [7]. The possibility that modification of ion homeostasis could be used to promote viral egress deserves further consideration.

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