Current Issues and Future Directions of Oncolytic Adenoviruses

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Oncolytic adenoviruses (Ads) constitute a promising new class of anticancer agent. They are based on the well-studied adenoviral vector system, which lends itself to concept-driven design to generate oncolytic variants. The first oncolytic Ad was approved as a drug in China in 2005, although clinical efficacy observed in human trials has failed to reach the high expectations that were based on studies in animal models. Current obstacles to the full realization of efficacy of this class of anticancer agent include (i) limited efficiency of infection and specific replication in tumor cells, (ii) limited vector spread within the tumor, (iii) imperfect animal models and methods of *in vivo* imaging, and (iv) an incomplete understanding of the interaction of these agents with the host. In this review, we discuss recent advances in the field of oncolytic Ads and potential ways to overcome current obstacles to their clinical application and efficacy.

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ONCOLYTIC ADENOVIRUSES ARE A NEW CLASS OF ANTICANCER AGENTS

Oncolytic replication–selective viruses are a new class of anticancer agents with great therapeutic potential.[1–4] The selective replication of the viruses in cancer cells amplifies the initial viral inoculum, leading to destruction of the infected cells by virus-mediated cytolysis. The viral progenies are thereby released and can spread through the tumor mass to infect neighboring cancer cells, resulting in self-perpetuating cycles of infection, replication, and oncolysis. Past cancer gene therapy clinical trials have defined major limitations of replication-defective vectors for cancer gene therapy; unable to infect the majority of the cells within a clinically presented three-dimensional solid tumor mass. Replication-selective viruses are designed to overcome such clinical limitations of cancer gene therapy by virus replication/spread whereas the restriction of their replication to tumor cells embodies the safety of oncolytic viruses for clinical usage.[2,3]

Although several oncolytic viruses have been identified to date,[5–7] replication-selective adenoviruses (Ads) based on human serotype 5 of species C possess a number of advantages.[1–4] Human serotype 5 Ads, which are associated with relatively mild diseases, are well characterized, their genomes can be manipulated with relative ease, and they can be purified to high titer.[2,8] Moreover, the long history of usage as replication-defective adenoviral vectors for cancer gene therapy has defined the strategies for cancer targeting, including targeting based on binding and infection as well as the strategies to restrict the replication of Ads to tumor cells.[1–4] The latter can be achieved via placing the expression of viral genes, most commonly the *E1A* gene, under the control of tumor- or tissue-specific promoters, or via the complete or partial deletion of viral genes required for replication in normal cells, but not in tumor cells.[1] In this sense, Ad is one of a few systems which are capable of concept-based design of the vector structure in the context of oncolytic viruses.

In 2005, State Food and Drug Administration in China approved mutation-based oncolytic Ad (H101; Sunway Biotech, Shanghai, P.R. China) as a drug for head and neck squamous cell carcinoma for local injection based on good responses observed in clinical trials.[9] This brings the hope that oncolytic Ads can be used in patients in the countries other than China.

REPLICATING ADS ARE PROMISING BUT HAVE SHOWN LIMITED EFFICACY IN HUMAN CLINICAL TRIALS SO FAR

In recognition of their therapeutic potential, replication-selective Ads have been rapidly translated into human clinical trials in patients with advanced cancer,[10,11] where their safety has been demonstrated. In this regard, a phase I clinical trial of an intraperitoneally administered replication-selective Ad has been conducted in patients with recurrent/refractory ovarian cancer.[12] In this trial, the maximum tolerated dose was not reached at 10^{11} plaque-forming units, and patients did not experience significant toxicity with this dose of administration.[12] Hence, there is a precedent for the safe use of replication-selective Ads in ovarian cancer patients.

However, in spite of their promise as selective cancer therapeutics, replicating Ads have shown limited efficacy in the clinical

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setting.[3] In this regard, there was no clear-cut evidence of clinical or radiologic response in any of the 16 patients with recurrent/refractory ovarian cancer who received an intraperitoneal replication–selective Ad in the phase I trial.[12] Similarly, phase I and II clinical trials in which patients with recurrent squamous cell carcinoma of the head and neck received direct intratumoral injection of a replicating Ad, ONYX-015, resulted in clinical benefit in <15% of cases.[13,14] Only when combined with standard chemotherapy did this oncolytic Ad cause an objective response (at least a 50% reduction in tumor size) in 19 of 30 cases, with 8 complete responses.[15,16] This indicates a need for implementation of novel strategies to improve the efficacy of replicating Ads while assuring safety in normal cells for clinical application in the treatment of patients with cancer.

EFFECTIVE INFECTION OF CANCER CELLS

As noted, there is no conditionally replicative adenovirus (CRAd) which shows clinical efficacy as a single therapy agent so far. This highlights the recognition that further augmentation of antitumor effects is the most crucial issue in CRAd development. Although Ad has been used for cancer gene therapy due to its exceptionally high *in vivo* transduction efficiency, many cancers (including gastrointestinal cancers, pancreatic cancer, ovarian cancer, and hormone-refractory prostate cancer) do not express Ad primary receptor (coxsackie adenovirus receptor, CAR).[2,3,17–19] Without a strategy for infecting target cells via CAR-independent pathways, achieving sufficient antitumor efficiency is difficult with the initial generation CRAd systems (**Figure 1a**). On this basis, various strategies have been developed to address CRAd infectivity. Specific strategies for Ad infectivity enhancement are:

Fiber modification

The Ad fiber region contains the "knob" domain which binds to the primary adenoviral receptor (CAR) on the surface of target cells. Because this position is the natural binding locale, a number of fiber-knob modifications have been endeavored to increase viral infectivity for CAR negative cancer cells. One of the most effective fiber modification is based upon infectivity enhancement via incorporation of an arginine–glycine–aspartic acid-4C (RGD-4C) motif into the HI-loop of the fiber-knob region[20,21] (**Figure 1b**). The RGD-4C motif is a partial peptide sequence of fibronectin identified by phage library screening. When it was incorporated into the surface exposed HI-loop of the fiber-knob region, the Ad vector with this motif showed CAR-independent infection of the target cells. Also, oncolytic Ads with this motif showed an augmented cytocidal effect in CAR negative cancer cell lines *in vitro* and *in vivo*.[18] However, identification of new peptide motifs for Ad modification is nontrivial. Most attempts to incorporate preidentified peptide coding sequence have failed because of a lack of production of fully assembled virus, or the incorporated motif did not show sufficient affinity to the binding counterpart on the surface of the target cells. Screening of the ligand in the form presented in the fiber-knob region is logical direction but there remains the issue of adequate library diversity.

Switching serotypes

Historically, Ad vectors have been derived based on subtype 2 or 5. This is the reason that CAR deficiency on the target cell is a major issue for Ad-based cancer gene therapy. Interestingly, other Ad serotype vectors do not necessarily use CAR as the primary receptor. For example, Ad35 uses CD46 for initial binding, and thus the infection is CAR independent.[22,23] There are basically two approaches for incorporating other subtypes' tropism into adenoviral vectors. One approach is to make a vector fully based on alternate subtype vectors (**Figure 1c**); the other is to design an Ad2/5-based vector with an alternate subtype's binding domain incorporated (chimeric) (**Figure 1d**). The first method has the advantage that all parts of the capsid consist of alternate subtype Ad proteins, thus the distribution is assumed to be completely the same as the parental virus. However, in this approach there is no guarantee that the CRAd replication processes after viral entry are as efficient as those of Ad2/5. In recognition of this fact, chimeric approaches are more frequently adaptable to oncolytic Ads, which require efficient post-transduction processes for efficient replication.[19,24,25]

Mosaic

Mosaic vectors are vectors with multiple binding moieties derived from different parental viruses and/or targeting peptides (**Figure 1e**). Such vectors not only embody a wider infectious range,

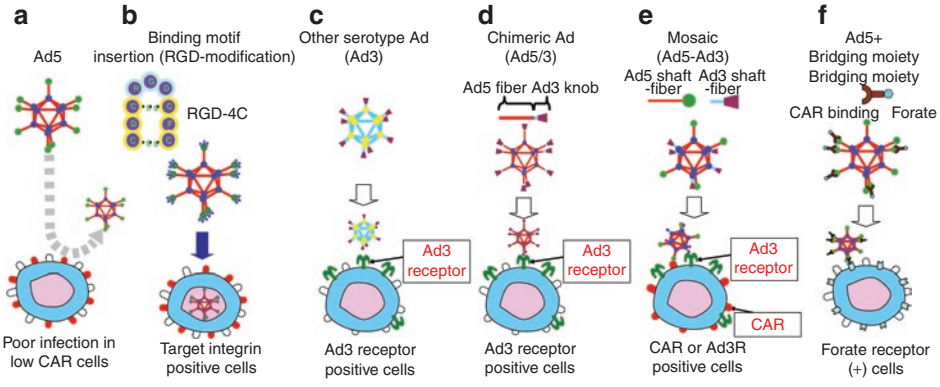

Figure 1 Modification of adenovirus (Ad) to achieve coxsackie adenovirus receptor (CAR)-independent transduction. To achieve CAR-independent transduction, several modification strategies have been employed in Ad. (**a**) Poor infectivity of CAR negative cells with conventional Ad system, (**b**) fiber modification, (**c**) switching serotypes, (**d**) chimeric, (**e**) mosaic, and (**f**) bridging molecule-based targeting (see detail in text).

due to multiple binding motifs, but also show higher infectivity compared to singly targeted vectors. Because enhanced infection is a crucial aspect of CRAd functionality and efficacy, such modifications have been incorporated into CRAds, which thereby show an improved cytocidal effect.[26] Such mosaic vectors can be derived by either pseudotyping, with 293 cells expressing different binding motifs, or by incorporating more than one fiber into the vector genome.[26,27] Because the progeny virus needs the same efficient binding, genetic incorporation is preferred in CRAds.

Bridging molecule-based targeting

This method can achieve the precise selectivity embodied by employing a high affinity/specificity antibody (Ab), or a specific binding motif for the target moiety expressed on the surface of the cells[28] (**Figure 1f**). Such bridging can be realized by anti-knob Ab fused with targeting ligands/Abs[28,29] or incorporation of immunoglobulin binding domains into the viral fiber.[30] Although this kind of targeting is functional only for initial entry, the targeting ability is high. Thus, there is a good possibility that such a bridging moiety can be applied for CRAds, especially for systemic administration. Although transductional targeting by these modifications is very useful for improving the efficacy of CRAd therapy, it does not provide sufficient levels of selectivity to the progeny viruses. This is because the transduction-based CRAd selectivity is currently imprecise and because efficient incorporation of the extrinsic targeting moiety into the intratumorally produced progeny virus is uneasy. At this time, bridging moiety-based targeting is used as a way to increase initial infectivity in CAR negative cancer cells,[29] or a way to deliver the systemically administered virus to the tumor locale.[31]

REPLICATION SPECIFICITY

The second key issue in CRAd development is a replication control mechanism for selectivity. Aforementioned various methods have been utilized for controlling viral replication in a tumor-specific manner. However, some replication control systems may not function as originally designed. For example, ONYX-015 was originally introduced as a virus that specifically replicates in cancer cells with mutation of the p53 tumor suppressor gene.[32] Although this virus was shown to be safe in early phase clinical trials, there are studies suggesting that the selectivity of this agent is based on p14ARF status in p53 functional cells.[33] Another report states that replication of the same virus does not depend on both p53 or p14ARF status.[34] Likewise, the CRAd agent AdΔ24 has also shown incomplete specificity, allowing replication in pRb intact cells in some experimental systems.[35,36] Thus, additional deletions have been performed to confer more stringent selectivity.[35–37] These approaches are designed because selectivity becomes particularly important in CRAds displaying an augmented antitumor effect, whereby sufficient clinical safety must be maintained. In addition, the fact that each tumor context may present different requirements for selectivity highlights the need to develop more variety of precise control mechanisms for future CRAd design.

In the process of the development of CRAds, strict control of selectivity has historically been considered the key issue relevant to developmental realization. However, wild-type Ad5 had been injected into cervical cancers in the 1950s, causing no severe toxicity.[38] More recently, agents without additional control mechanisms (ING007) have been approved for phase I clinical trial.[39,40] These are based on the data of intrinsic cancer selectivity of wild-type Ad5. However, in the context of tropism-modified Ads, the US Food and Drug Administration still has concerns vis-à-vis replicative selectivity due to possibility of infection of a wider range of normal cells as a consequence of infectivity enhancement.

With respect to the achievement of replicative selectivity, two general strategies have been endeavored. The first type is based upon mutations in the viral genome which are essential for viral replication in normal cells but can be selectively compensated by particular defects in cancer cells (**Figure 2a**). In this regard, the dl1520 (or ONYX-015) Ad lacks the E1B region and was originally designed to achieve selective replication in cancer cells with mutated p53.[32] AdΔ24 has a mutation in the E1A region which theoretically restricts replication to cancer cells with mutated pRb.[41] The second type of CRAds relies on cancer-specific, promoter-controlled transcription of the E1 region (**Figure 2b**). Because the E1A protein is necessary for the replication of Ad, these viruses

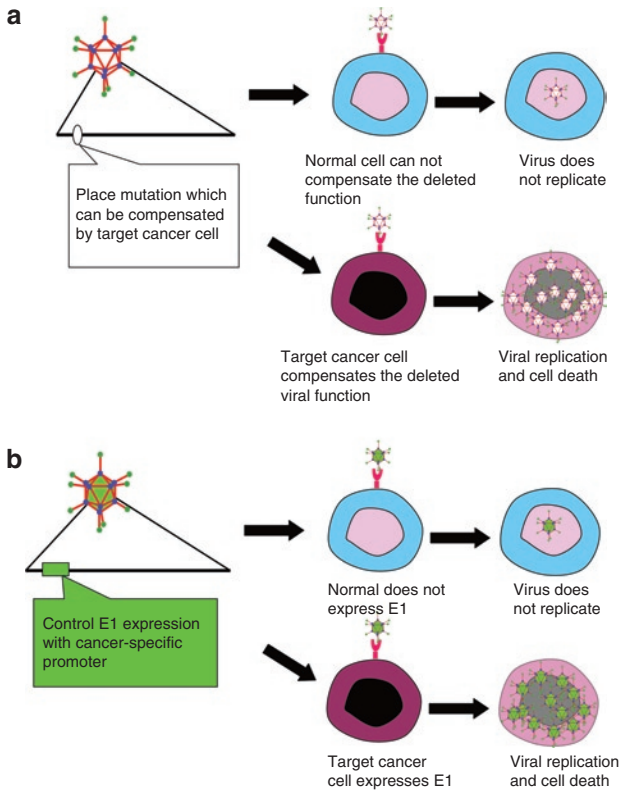

Figure 2 Control mechanisms of oncolytic adenovirus. (**a**) Deletion-type conditionally replicative adenoviruses (CRAds): this type of CRAd has a mutation/deletion in a region crucial for viral replication. While cancer cells possess the cellular environment to compensate the function of the deleted viral gene, normal cells do not have that capability. For example, ONYX-015 (dl1520) and AdΔ24 were designed to replicate only in p53 and pRb mutated cells, respectively. (**b**) Selective promoter-based CRAds (*i.e.*, RGDCRAdCOX2F, CN706, CV739): a tumor/tissue-specific promoter controls the expression of viral genes crucial for replication. As a result, the virus can replicate only in cells in which the promoter is active. By using a promoter with a tumor-ON/normal cell-OFF profile, the replication can be limited to cancer cells.

can replicate only in cells where the controlling promoter is active. In this regard, CN706 has a prostate-specific antigen promoter–driven E1 expression cassette which enables selective replication in prostate cancer cells in an androgen-dependent manner.[42]

As an emerging strategy for CRAd replication control, post-transcriptional control can be also used. Micro-RNAs are short RNAs which are expressed in the cells and determine many aspects of the cell characteristics. By placing micro-RNA binding sequence into the context of Ad E1A, replication can be restricted to the target cells (*e.g.*, cancer cells).[43] Also, the 5′- or 3′-untranslated region placed on E1A can embody cancer specificity.[44,45]

LATERAL SPREAD

CRAd infection and its productive replication would ideally result in the dissemination of progeny virions throughout the target tumor.[1,4] This local amplification of viral inocolum constitutes the basis of the expected multiplicative effects of CRAds, which is the definitive difference of CRAds from nonreplicative Ad agents.[2,3] Full exploitation of this amplified local viral mass, however, requires effective lateral spread of the virus via infection of the neighboring cancer cells. In fact, both cellular and tissue barriers render this process as a limiting factor in CRAd physiology.[1,4] On this basis, strategies have been proposed to enhance target cell killing by CRAds in order to enhance effective escape and release of progeny virions from tumor cells. Such strategies have sought to facilitate the apoptotic killing function of CRAd action by configuring into the Ad genome human tumor suppressor genes, such as p53,[46,47] or viral genes involved in the native process of target cell senescence, such as the Ad death protein.[48,49] Each of these approaches has yielded so-called "armed" CRAds with enhanced potencies exhibited in model systems. Other strategies for CRAd arming have exploited toxin genes previously studied in the context of replication-defective Ad-based molecular chemotherapy in cancer gene therapy schemas. Cautionary reports have highlighted that selected antitumor genes may actually operate at cross-purpose with Ad replicative physiology, yielding armed CRAd agents with reduced potency compared to their unarmed parental counterparts. On this basis, recent arming strategies tend to exploit encoded transgenes with antitumor activities with potential synergy with CRAd replicative physiology. These approaches have sought to utilize antiangiogenesis genes[50,51] and other factors directed at tumor microenvironment biology.[52] In addition, immunostimulatory genes have been configured into CRAds to induce antitumor immune reaction.[53]

MODEL SYSTEMS

In addition to vector engineering, valid *in vivo* experimental systems need to be developed for further understanding CRAd functionality. In particular, a convenient *in vivo* experimental system for the analyses of CRAd replication/toxicity and virus–host interaction is urgently needed. To date, most *in vivo* experiments have been performed with human cancer cell xenografts in immunodeficient mice. However, the stringent species selectivity of adenoviridae replication does not permit human Ad to replicate in most rodents cells including mice and rats. This biology greatly limits the ability to conduct virus replication–related studies in one of the most useful experimental animals. Cotton rat and Syrian hamster permit productive human Ad replication,[54] however, it is not yet clear how closely viral replication in this system resembles that in humans. The fact that this is the only small animal model system permissive for human Ad replication highlights the importance of this model especially in the context of toxicological studies.[39] In addition, syngeneic models have been proposed to better understand the biology of replicative Ad in the matched host settings. One current model is based upon hamster cancer cell line syngeneic graft in Syrian hamsters.[55] Another approach is to employ conditionally replicative canine Ads to treat spontaneous dog osteosarcoma.[56,57] This unique model would provide valuable information about an oncolytic agent in its natural host, and such data would be uniquely translatable to human context. However, experiments with nonhuman, nonmouse models still have relevance vis-à-vis analyzing host-specific phenomena *e.g.*, immunity.

IMAGING

The development of a noninvasive monitoring system to track treatment is another challenge. Although the ultimate goal of CRAd therapy is to achieve an antitumor effect, determination of CRAd functionality requires interval end-point assays to monitor the progress of treatment. These assays should be informative yet minimally invasive and would also be valuable for maintaining safety in clinical trials.[2] For example, if the expression of the TK toxin gene is detected in normal organs, administration of the prodrug could be halted to prevent adverse event. In the context of CRAds, such therapy monitoring has heretofore been performed by immunoblotting or immunohistochemistry of biopsy specimens.[11] Although biopsy examination is informative, this procedure is considerably invasive. Also, the data obtained reflect only one time point when the specimen was taken: the monitoring the dynamic replication/spread of CRAds would require biopsies at multiple time points. *In vivo* imaging has been pursued to monitor transgene expression after vector administration.[58–60]

Initial localization of the vector can be determined by conventional labeling of the viral capsid with tracers (*e.g.*, fluorophore, I^{131}), or placing a replication-independent expression cassette of noninvasively detectable marker gene in the CRAds.[61] However, in the latter case, the expression of the marker increases along with viral genome copy number upon viral amplification, and thus results in the representation of viral replication.

Imaging of viral replication requires the marker gene expression in a replication-dependent manner. When we placed the marker gene into the E3 region of CRAds under the control of major late promoter, the reporter gene was expressed only when the virus is replicating.[62] This is because major late promoter is closely linked to the Ad replication cycle. We also placed the reporter as a fusion protein with the viral capsid protein pIX, which is exposed to the outside of the virion.[63] The capsid of the virus with pIX-RFP fusion protein was fluorescently labeled. Interestingly, the virus showed a signal corresponding to viral replication. In this instance, the representation of the replication status in this vector is linked to the property of the pIX promoter in activation after early gene expression.

At this time, optical imaging methods which are most commonly used for *in vivo* imaging cannot be utilized directly for

Table 1 Typical radionuclide-based imaging reporters for gene therapy application

Reporter	Function	Typical imaging reagent
Thymidine kinase (TKsr39)	Phosphorylation of thymidine analogs (*e.g.*, ganciclovir) and permit radionuclide accumulation in the cells	[^{18}F]FHBG
Dopamine receptor	Exist on the surface of target cells and permit binding of radiolabeled dopamine	[^{18}F]FESP
Somatostatin receptor type 2	Exist on the surface of target cells and permit binding of radiolabeled specific ligand	^{99m}Tc-P829 (Neotect)
Sodium iodine symporter	Transport iodine and other anions (*e.g.*, TcO_4^-) and increase their cellular accumulation	$^{131}I^-$ $^{99m}TcO_4^-$

human application. Luciferase-based imaging requires substrate injection upon imaging and detection of fluorescence. In addition, this method is limited by issues of detection sensitivity and background autofluorescence. Thus, the radionuclide-based imaging reporters have greater possibility for human application due to their ability for imaging of tumors, established imaging protocols, as well as established safety profiles.[64,65] **Table 1** shows the most commonly used radionuclide-based imaging techniques in conjunction with gene therapy. The realization of such replication monitoring strategy is awaited for safer and more efficient CRAd therapy.

SYSTEMIC VERSUS LOCAL THERAPY

The overwhelming majority of CRAd applications, both in model systems and in early phase human clinical trials, have been for the context of local disease. This reflects the fact that Ad administered via the systemic route localizes principally to the liver. The consequences of this biology are that liver localization may limit desired delivery of Ad to tumor targets and may also elicit hepatotoxicities.[28] This biology has thus restricted the employment of Ad for any clinical context involving systemic delivery schemas. In addition, preformed Abs as the result of community acquired infection with parental Ad or prior treatment with similar vectors could neutralize Ad administered in this manner, confounding the goals of effective agent delivery to tumor cells.[66] Nonetheless, some early phase human clinical trials have administered CRAd agents via the vascular route without any noted untoward effects, indicating that the pre-existing Ab does not compromise the safety of the CRAds. Recently, various targeting strategies have realized effective tumor transduction with decent selectivity with modified Ads delivered systemically.[2–4] Some of these targeting methods allow effective mitigation of viral particle sequestration in the liver.[67] In addition to the efforts to eliminate the natural tropism by viral capsid modification, a number of efforts have been reported to avoid the contact of the vector with unwanted cells by making the structure stealth. Coating with polymer or polyethylene glycol is reported to not only evade the immune system but also increase the delivery to the tumor locale.[68,69] Alternatively, usage of various cells as a vehicle for the delivery of oncolytic Ad has been exploited as a way to increase tumor targeting upon systemic delivery.[70,71]

The lack of effective treatments for disseminated disease suggests the desirability of application of Ad-based virotherapy for the metastatic disease context. Particularly, CRAd-based therapeutics require far lower initial tumor transduction for its functionality compared to those with nonreplicative vectors thanks to local multiplication of the vector. Thus, the aforementioned improvements of targeting have made systemic CRAd delivery more realistic.

LIVER SEQUESTRATION AND COAGULATION FACTORS

As widely known, Ad released into systemic circulation sequesters in the liver in mice. Initially, such distribution was thought to be mediated by high CAR expression of liver parenchymal cells. However, analyses with CAR binding–ablated viruses and/or penton base RGD-deleted viruses indicated that neither CAR binding nor the integrin binding can fully explain liver sequestration.[72] Cationic repeat (KKTK) in the fiber-shaft region was also suggested for explanation. However, analyses with chimeric Ad5 with Ad35 fiber suggest that liver distribution can be observed without KKTK in the shaft and that platelet depletion virtually eliminated the liver sequestration.[73] More recently, Ad hexon particularly hypervariable region 5 has been shown to be crucial for viral binding to a coagulation factor (factor X).[74–76] This indicates that the liver sequestration observed in mice may be altered by mutating hypervariable region in adenoviral hexon protein. However, there is still a question whether this understanding can be generalized to other species, including humans, as mice are known to show extremely high liver sequestration of human Ad compared to other mammals.

HOST IMMUNOLOGICAL REACTIONS

It is widely recognized that most adults have neutralizing Ab against most common vector strains (Ad2 or 5). Thus, pre-existing immunity is understood to be a major issue for CRAd functionality. However, clinical trials based upon local administration of CRAd in prostate cancer have shown no correlation between neutralizing Ab levels and the effect on prostate-specific antigen.[11] The same phenomenon was observed in syngeneic tumor models in immunocompetent hamsters.[77] This recognition suggests that pre-existing neutralizing Ab may not be a major factor affecting therapeutic outcomes in CRAd local administration. However, pre-existing Ab may still be a major obstacle in the context of systemic administration of CRAd because Ab may neutralize the CRAds before it reaches the target site.

The effect of cellular immune response to antitumor effect is supposed to be affected by a delicate balance between induction of antitumor response and elimination of virus itself.[78] A recent publication indicates that the enhanced *in vivo* antitumor effect in the combination of E3B-deleted Ad (dl309) and paclitaxel was observed only in immunocompetent mice,[79] indicating the interaction of viral function and host immune system.

On the other hand, innate immune response has been a major issue in Ad vector system in general. This is particularly valid as the one and only lethal adverse effect reported with Ad vector is understood to be due to innate immune response showing cytokine storm. Recently, Toll-like receptor (TLR) has been reported to

play a major role in innate immune response against DNA viruses including Ads via TLR9 as a response to double-strand DNA introduction.[80,81] However, Di Paolo *et al.* recently reported that there is another cascade independent of TLR9 or NLRP3 inducing innate immune response against double-stranded DNA.[82] The IL-1α-mediated anti-Ad response required a selective interaction of virus RGD motifs with macrophage β3 integrins. So far, oncolytic Ad development has been taking advantage of the low requirement of initial administration dose as a way to avoid innate immune response. In addition, the above-mentioned elucidation of the antiviral responses may lead to vector design which can efficiently mitigate the innate immune response issue in this field.

A variety of immunomodulators have been endeavored in oncolytic virus systems including CRAds, and cyclophosphamide is the most widely tested in animal models.[77,83] In syngeneic tumor models in immunocompetent hamster, cyclophosphamide induced prolonged local viral replication and enhanced the antitumor effect.[84] In other oncolytic virus models, other mechanisms of enhancement (*e.g.*, suppression of regulatory T cells) are reported for cyclophosphamide.[83,85] In this regard, the application of immunomodulators upon CRAd therapy will continue to be an important subject for enhancing the effect of CRAd therapies.

TOWARD CLINICAL USAGE

As mentioned earlier, H101 (E1b55K-deleted oncolytic Ad) has been approved as a drug in P.R. China.[9] In the United States, there are several clinical trials with oncolytic Ads including ONYX-015 (Onyx Pharmaceuticals, Emeryville, CA) with similar structure, but none of them has been approved for the treatment of patients. The response to H101 plus chemotherapy in phase III was 79% (41/52),[9] which is significantly higher than ONYX-015 phase II data.[14] Although there is some discussion doubting the fairness of tumor response evaluation,[86] such points will be elucidated through the accumulation of cases in real clinical settings. Another possible difference is the refractoriness of the disease between the studies. While ONYX-015 trial was only for refractory patients, the phase III H101 trial includes many patients without prior treatment history. This may mean that the patients with earlier disease may be a better target of oncolytic virus therapy compared to those with advanced disease. The other possible branching factor may be the cost for phase III and IV studies. Practically, the costs of later phase clinical studies are astronomical, and thus many small to mid-size pharmaceutical companies are forced to reduce the number of research projects when one candidate is entering phase III. This prioritization issue might have practical impact on the decision-making for later phase clinical studies.

OTHER CONSIDERATIONS

A cell population responsible for resistance to chemotherapy and disease relapse, called cancer stem (-like) cell, has been gathering a lot of attention. This population shows a low proliferation rate. Ad which shows high transduction in nonproliferating cells is a suitable choice of vector for that population.[87,88]

Recently, many new vectors as well as improvements of existing oncolytic virus systems have been reported.[89] Thus, it will be necessary to fairly compare the effect of various vectors in a reasonable *in vivo* system. Comparing them in humans is ideal because all vectors are intended for usage in humans, although impractical. In addition, it is not that easy to compare different vectors *in vivo* models because the tropism of each virus replication is highly stringent. At this time, finding acceptable *in vivo* experimental models for comparison of different viral system is very difficult.

Kuhn *et al.* reported generation of more potent chimeric viruses by coinfection of multiple serotype vectors.[90] This method has a potential to generate a lot of vectors with novel tropism, but the issue of long-term stability may need to be addressed for future clinical usage of resultant vectors.

From a practical vector production stand point, vector manufacturing of oncolytic Ads requires special consideration. Compared to E1-deleted vectors, the oncolytic Ad with intact E1 region has a much higher possibility for recombination generating the left end structure of wild-type Ad. Although the Food and Drug Administration accepts a little bit higher level of contaminating wild-type vector for oncolytic Ads compared to that in replication-deficient vectors, the effort to reduce wild-type contamination is crucial. We have been using target cancer cell lines without *E1* gene (*e.g.*, A549 lung cancer cell for cyclooxygenase-2 promoter–driven virus) for amplification and getting good yield at high quality.

CONCLUSION

In this review, we discussed advances in the field of oncolytic Ads as well as current obstacles and the directions for overcoming them. Oncolytic Ad is an efficient and interesting material for cancer therapeutics development because accumulated knowledge and technique enable rationale-driven vector design. We believe that future advances in adenovirology and its application will further advance the field of oncolytic Ad toward full realization of the potential of oncolytic Ads.

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