

Minireview

Conditionally Replicative Adenoviruses for Ovarian Cancer Therapy

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Abstract

The purpose of this review article is to present a logical rationale for the investigation of conditionally replicative adenoviral vectors for the treatment of ovarian carcinoma. A medline database search was performed to identify relevant articles in the English language for the years 1966 to present. The key words used included replicative adenovirus, conditionally replicative adenovirus, transcriptional targeting, replication selective adenovirus, and "ONYX." A total of 89 references were identified and reviewed. Each reference was reviewed for relevance to clinical translation of conditionally replicative adenoviral vector therapy for ovarian cancer. Data from current clinical trials would suggest that potential obstacles for effective replicative viral therapy of ovarian carcinoma include efficient tumor cell infection, restrictions of the cell surface coxsackie and adenovirus receptor, rapid clearance of vector in the ascites environment, tumor cells specificity, and limitations of current findings of clinical trials. The articles were, therefore, evaluated and included if they addressed these shortcomings. Current data would suggest that advanced generation conditionally replicative adenoviral vectors will soon be available for clinical trials in ovarian cancer. Ovarian cancer, because of expression of targetable receptors, transducible cells, and containment within the i.p. cavity, represents a solid tumor suited uniquely for investigation with advanced generation conditionally replicative adenoviral vectors.

Introduction

Ovarian carcinoma is the fourth leading cause of cancer death in the female population and the most fatal gynecological malignancy. It is estimated that in 2001, there were 23,400 new cases of ovarian cancer, and ~13,900 women succumbed to this disease (1). Attributable primarily to a lack of effective screening strategies and vague signs and symp-

toms in early stage disease, 70% of women will present with stage III or IV cancer at the time of initial diagnosis. Despite advances in surgical technique and modern chemotherapy, the long-term survival for advanced ovarian carcinoma has remained ~15–30% over the last 20 years. Clearly, more effective treatment strategies are needed for this disease.

Genetic-based therapy represents a novel investigational approach to the treatment of ovarian cancer. However, the inability to achieve specific and efficient transfer of a therapeutic or cytotoxic genetic sequence to target cancer cells represents a major limitation in allowing practical clinical translation of cancer gene-based approaches. In this regard, results from preliminary human clinical gene therapy trials for ovarian cancer have uniformly demonstrated extremely limited gene transfer efficiencies of current generation vector systems. Consequently, tumor cell modification or death is rarely achieved to the level required to result in clinically significant effects. As a result, intensive efforts are underway toward the identification of novel and more efficient vector strategies that may lead to enhanced tumor eradication.

These efforts are consistent with the NIH (Orkin-Motulsky Report) that notes, "To confront the major outstanding obstacles to successful somatic gene therapy, greater focus on basic aspects of gene transfer and gene expression within the context of gene transfer approaches is required. Such efforts need to be applied to improving vectors for gene delivery, achieving tissue-specific and regulated expression of translated genes, and directing gene transfer to specific cell types." The utilization of potentially targetable replicative viral systems represents a novel genetic-based therapy approach consistent with this goal.

Replicative Viral Vector Systems for Cancer Therapy

In a therapeutic strategy using replicative viral systems, target tumor killing by the viral agent is achieved as a direct consequence of viral replication (2). This concept of "viral therapy" was inspired early in this century by the observation of occasional tumor regressions in cancer patients suffering from viral infections (3). Indeed, an early clinical trial in cervical carcinoma was carried out after the isolation of the adenovirus in 1953 (4). In this early trial, initial tumor regression was often observed after injection with adenovirus, but no sustainable responses were noted (4). A lack of sustainable therapeutic efficacy has led investigators to dismiss viral therapy until more recently when molecular biological techniques have allowed for greater manipulation of viral particles, resulting in new potential therapeutic agents.

To this end, an ideal viral agent would thus possess two logical attributes: (a) such virus must have the capacity to infect target cells *in situ*. Thus, a level of stability in the *in vivo* context is mandated to achieve an effective initial inoculum.

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This is particularly important in the context of i.p. delivery of therapeutic viral agents in patients with ovarian carcinoma. Elkas *et al.* (5) and Blackwell *et al.* (6) have identified inhibitory antibodies present in malignant ascites that drastically diminish the efficacy with which adenoviral mediated gene transfer can occur. Furthermore, such stability in the *in vivo* context would be critical for allowing replicating viruses to infect laterally, a new process for realizing effective amplification; and (b) the viral agent should possess a relative propensity for replication in tumor *versus* nontumor cells. Thus, a useful viral agent would be well characterized in terms of entry biology, and replicative physiology, such that these steps might be modified to achieve the desired tumor cell specificity. Specifically, modulations of viral tropism, by either alteration of the initial attachment/entry steps or modification of the functional aspects of viral genome replication, offer a means to achieve such specificity.

Use of Adenoviral-based Vectors for Cancer Gene-based Therapy

With respect to candidate replicative viral agents, adenoviruses possess many of the relevant attributes recommending their utility in this context. In this regard, adenoviral vectors have been used extensively for a variety of gene therapy applications (7). Adenovirus has exhibited an unparalleled efficiency, allowing effective infection of target cells in the context of *in vivo* gene delivery (7). Additionally, the cellular entry pathway of the virus has been studied extensively, resulting in strategies to enhance viral tropism for target tumor cells.

It has been reported that coxsackie B virus and adenovirus serotypes 2 and 5 share a common receptor, which has been designated CAR² (8). The initial high-affinity binding of Ad2 and Ad5 to the primary cellular receptor occurs via the knob domain of the fiber (9, 10). After knob-mediated attachment to the cell surface, the next step in infection by Ad2 and Ad5 is internalization of the virion by receptor-mediated endocytosis potentiated by the interaction of Arg-Gly-Asp (RGD) peptide sequences in the penton base with secondary host cell receptor integrins (11). After internalization, the virus is localized within the cellular vesicle system, initially in clathrin-coated vesicles and then in endosomes. The virion then localizes to the nuclear pore, and its genome is translocated to the nucleus. On the basis of this understanding of adenoviral biology, investigators have endeavored to abolish native tropism. Alterations in native tropisms are critical when considering utilization of adenovirus as ovarian tumor cell vectors. Our earlier studies have demonstrated that ovarian cancer cells are profoundly deficient in CAR receptor, resulting in a relative resistance to adenoviral infection (12). Novel tropism is then introduced by retargeting the adenovirus to a receptor common to ovarian cancer cells. As such, we have

explored the utility of the HI loop for incorporation of targeting ligands to allow modification of adenovirus tropism (13).

Genetic retargeting of the adenovirus may have significant implications when applied to the clinical scenario of ovarian cancer and replicative systems. As noted previously, these replicative adenoviral systems must have *in situ* stability to allow lateral infection to occur and demonstrate preferential infectivity of target tumor cells, thus, preserving the therapeutic index. Specifically, preclinical testing of an RGD tropism-modified adenoviral vector has consistently demonstrated CAR-independent gene transfer to primary ovarian cancer cells that is two to three orders of magnitude over that observed with an unmodified adenoviral vector (12). Because integrins have been shown to be overexpressed by various epithelial tumors, vector targeting to these cell surface receptors can provide a means to achieve CAR-independent gene transfer. Moreover, the RGD-modified adenoviral vector exhibits preferential gene transfer to primary ovarian cancer cells when compared with human mesothelial tissue (12).

Preliminary evidence has suggested that adenoviral infection may be inhibited significantly by the presence of preexisting IgG in human ascites (5, 6). Blackwell *et al.* (6), therefore, hypothesized that an Arg-Gly-Asp (RGD) peptide sequence inserted into the fiber knob domain of the adenovirus may confer "immune privilege" to this viral construct as this modification encodes for integrin receptors ubiquitous in human tissues. No inhibition of gene transfer efficiency was observed in the ovarian cancer cells transfected with the RGD-modified adenoviral vector in the presence of ascites when compared with the unmodified adenoviral vector (6). Thus, genetically induced infectivity enhancements may be of critical importance for a replicative system where vector stability in the i.p. environment is crucial.

Conditionally Replicative Adenoviral Vectors

Initial attempts to derive CRADs focused on the achievement of tumor selective replication. One such strategy has been the generation of type I conditionally replicative adenoviral vectors, which are targeted to biological factors modified in cancer cells. One such attenuated virus was developed to replicate only in cells lacking the cell cycle control protein p53 (14). This virus, dl1520 (Onyx-015), has a deletion in the E1B-M, 55,000 gene product, which is responsible for p53 binding and inactivation. Therefore, in theory, this deletion mutant would be unable to inactivate p53 in normal cells and would, consequently, be unable to replicate effectively. Alternatively, cancer cells lacking functional p53 would be sensitive to viral replication and subsequent oncolysis. Initial studies carried out with this attenuated adenovirus showed sensitivity of p53 mutant ovarian cancer cell lines to cytopathic effect. In addition, tumor regression was achieved in murine xenograft models (15). Subsequent studies by Gooch *et al.* (16) and Turnell *et al.* (17), however, have determined that actual specificity of viral replication of dl1520 is not because of the absence or presence of p53 but is based on the timing of viral replication in tumor cells. Thus, although the initial concept of targeting replication to the presence of a functional p53 gene was not realized with this virus, empiric efficacy in tumor treatment has been demonstrated (18).

² The abbreviations used are: CAR, coxsackie and adenovirus receptor; CRAD, conditionally replicative adenovirus; TSP, tumor-specific promoter; PSA, prostate-specific antigen; SLPI, secretory leukoprotease inhibitor; TK, thymidine kinase.

Another way to achieve tumor-specific adenoviral replication is to take advantage of altered cell cycle regulatory functions that occur in tumor *versus* normal tissue. In normal tissues, the G₁-S phase checkpoint is intact; therefore, S phase induction and viral replication are resisted. In almost all cancer cells, this checkpoint is lost as a result of any number of deletions or mutations; one such deletion is that of the *RB1* gene (19). With this in mind, an adenovirus was created that carries a deletion of the retinoblastoma gene (*Rb*) binding site of E1A (20). Because of this deletion, the mutant adenovirus, designated Ad5-Δ24, cannot overcome the G₁-S phase checkpoint in quiescent cells as effectively as wild-type virus. Consequently, Ad5-Δ24 is unable to replicate and spread in normal tissues, thereby, conferring a favorable therapeutic ratio to quiescent normal tissues. This virus has demonstrated efficacy in *in vitro* experiments of glioblastoma (21). In addition, a virus with the same functional deletion, designated dl922-947, replicates and lyses a broad range of cancer cells with irregularities in cell cycle checkpoints (22). Furthermore, i.v. administration reduces the incidence of metastases in a breast tumor xenograft model. Although this mutant demonstrates reduced S phase induction and replication in nonproliferating normal cells, the virus is still capable of replicating in proliferating normal cells which could prove problematic when translating this vector into the clinic; therefore, specific viral replication in tumor remains to be achieved with type I CRADs.

Potential TSPs for Use in CRADs for Ovarian Cancer

An additional promising strategy to confer specific oncolytic activity to CRADs is through the use of TSPs, referred to as the type II CRAD. In this approach, heterologous transcriptional control regions, or promoters, are used to restrict replication of the adenovirus to tumor. This has been accomplished by placing an essential adenoviral gene under the control of a TSP. The observation that levels of PSA are elevated in the prostate of individuals with prostate cancer led to the development of adenoviral vectors with the transcriptional promoter sequences of the *PSA* gene configured in them to regulate E1A expression (23). In mouse xenograft models, this replicative adenovirus eradicated large PSA-expressing tumors and abolished PSA production (23). Another type II CRAD used for cancer therapy has used the sequences that drive the expression of the hepatocellular carcinoma marker, *α-fetoprotein*, a gene that is singularly expressed in dividing hepatocytes and hepatocellular carcinoma (24). This strategy of incorporating TSP elements within the adenoviral genetic structure appears to be very promising in its ability to enhance the therapeutic ratio of CRAD.

Several candidate promoters have been analyzed in gene therapy studies for specific transcriptional control in ovarian cancer cells, which may hold promise in the context of future CRAD development for ovarian cancer. One promising TSP that has been investigated in an ovarian cancer context has been the *SLPI* promoter. The *SLPI* gene has been shown to be expressed in ovarian carcinoma cells, as well as lung, breast, oropharyngeal, bladder, endometrial, and colorectal carcinomas. The *SLPI* promoter has been used in a plasmid

construct to direct the expression of herpes simplex virus TK in a variety of carcinoma cell lines, including SKOV3 ovarian cancer cells, achieving specific cell killing (25, 26).

Another candidate ovarian-specific TSP includes the *human α folate receptor* gene promoters. High affinity folate receptors are expressed in normal ovaries and in the vast majority of ovarian adenocarcinomas. The *human α folate receptor* gene contains two tissue-specific promoters, P1 and P4. Goldsmith *et al.* (27) constructed a recombinant adenovirus that harbored the P1 promoter driving the luciferase gene. Several ovarian carcinoma cell lines were infected, and correlation was demonstrated between folate receptor levels and reporter gene expression.

In addition, the *MUC1/DF3* gene promoter, *MUC1*, has been used to drive the proapoptotic *bax* gene and has shown specific cell killing in ovarian cancer cell lines and in a murine model of ovarian cancer (28). The *MUC1/DF3* gene encodes the polymorphic epithelial mucin protein, which is expressed in glandular epithelial cells in human tissues. The protein is overexpressed in most carcinomas, mainly because of transcriptional up-regulation. The cancer associated mucin, although very similar to its normal counterpart, has a distinct antigenic profile (29). It has been reported that the epitope is expressed in ovarian adenocarcinomas, including the serous, mucinous, endometrioid, clear cell, and undifferentiated subtypes (30). Regulatory regions of this promoter, with distal enhancer elements, have been identified (31). Ring *et al.* (29) generated recombinant retroviruses containing the *herpes simplex virus TK* gene under the control of the *MUC1* promoter and showed increased ganciclovir sensitivity in pancreatic and breast carcinoma cell lines.

The *L-plastin* gene product is a member of the actin-binding proteins and is highly expressed in most human epithelial cancer cells (32). The *L-plastin* promoter has been incorporated in a replication deficient adenovirus, driving the *Escherichia coli LacZ* gene. This construct has been tested in a variety of cell lines, including ovarian cancer cells and mesothelial cells (32). The *L-plastin*-driven transgene expression appeared to be restricted to the ovarian carcinoma cells, while sparing the mesothelium (32).

Casado *et al.* (33) have investigated the utility of midline and cyclooxygenase-2 gene promoter regions to function as TSP regions in ovarian cancer. These authors demonstrated expression of reporter genes under the control of these promoters in established and primary ovarian cancer cells. In *in vivo* xenograft experiments, using i.p. administered SKOV-3 ovarian cancer cells demonstrated marked decrease in mesothelium-related expression of reporter genes relative to tumor implants. Similar *in vivo* cytotoxicity was also observed when a cyclooxygenase-2 promoter-driven TK construct was compared with the standard cytomegalovirus promoter-driven TK gene construct (33).

Thus, there appears to be several candidate promoters poised to be incorporated into type II CRAD vectors for future clinical trials investigating novel gene therapy approaches in patients with ovarian cancer. The challenge for these trials will be in determining whether controlling transcription via TSPs will enhance the therapeutic index in patients.

A Candidate Conditionally Replicative Adenovirus for Ovarian Cancer Gene Therapy

Unmodified CRADs have been advanced into early clinical trials for head and neck, pancreas, ovary, colorectal, lung, and oral carcinomas (34). Neumanaitis *et al.* (35) reported initial Phase I data using the ONYX-015 CRAD in patients with metastatic solid tumors. The ONYX-015 was infused i.v. at escalating doses of 2×10^{10} to 2×10^{13} particles via weekly infusion within 21-day cycles (two cycles maximum) in 10 patients with advanced solid carcinomas metastatic to the lung. No dose-limiting toxicity was observed in this cohort of patients. Evidence of replication, by PCR evidence of increased viral genome concentration, was observed in 3 of 4 patients receiving $\geq 2 \times 10^{12}$. After a Phase I trial where no untoward toxicity was observed, a Phase II study of refractory head and neck tumors, 2×10^{11} particles were administered intratumorally for 5 consecutive days or twice daily for 2 consecutive weeks (18). The treatment was well tolerated, and a 14% response rate was observed, whereas 41% experienced stable disease. These clinical studies develop the rationale for the potential utility of CRAD for use in patients with metastatic solid tumors.

When considering the ideal CRAD for ovarian cancer genetic therapy, several conditions should be considered. Given the low CAR environment known to exist on the cell surface of primary ovarian cancer cells, a mechanism of enhancement of infectivity should exist. In addition, modifications to the viral receptor mechanism should be made that attempt to improve tropism to tumor cells relative to normal cells. Finally, the virus should be stable in the peritoneal/ascites environment encountered in patients with advanced ovarian cancer so as to allow lateral transmission of the replicative oncolytic adenovirus. To address these issues, Suzuki *et al.* (21) have reported the development of an infectivity enhanced CRAD for use in cancer gene therapy. These investigators used the previously described genetic modification of the fiber knob to incorporate the Arg-Gly-Asp (RGD) sequence, which has conferred enhanced gene transfer to low CAR-possessing cells through interaction with cell surface integrins overexpressed on cancer cells into the Ad5- Δ 24 backbone. Preclinical evaluation of this virus (Ad5- Δ 24-RGD) has demonstrated *in vitro* cytotoxicity in cultured lung cancer cells. Moreover, an enhanced oncolytic effect was demonstrated in A549 xenografted tumor nodules in nude mice using a single injection of Ad5- Δ 24-RGD when compared with unmodified Ad5- Δ 24.

The Ad5- Δ 24-RGD virus capitalizes on the finding that the p16-RB pathway is defective in many human cancers, including ovarian cancer (36–38) This potential therapeutic is therefore undergoing preclinical testing in the context of ovarian carcinoma. To date, preliminary data suggest that the Ad5- Δ 24-RGD reagent has comparable oncolytic activity to wild-type virus and replicates efficiently in primary ovarian cancer cell cultures. More importantly, in a murine model of i.p. ovarian cancer, all mice injected with the Ad5- Δ 24-RGD

demonstrate complete eradication of intra-abdominal tumor.³

The Ad5- Δ 24-RGD virus is an attractive novel agent in the context of ovarian cancer given the enhanced gene transfer to ovarian cancer cells observed with RGD-modified adenovirus, the overexpression of cell surface integrins known to be present on the surface of ovarian cancer cells, and the stability of RGD-modified adenoviruses in the presence of inhibitory antibodies in ovarian cancer-related human ascites.

Conclusion

The application of gene therapy as a investigational endeavor for the treatment of malignancy is developing rapidly. The utilization of replicative adenovirus to potentiate cytopathic effects in tumor cells represents an exciting new approach for solid tumor therapy. Moreover, advances in our understanding of viral biology have led to genetic manipulations which enhance viral tropism, gene transfer efficiency, tumor-specific replication control, and stability in hostile ascitic environments. Ovarian cancer, because of expression of targetable receptors, transducible cells, and containment within the i.p. cavity, represents a solid tumor suited uniquely for investigation with advanced generation conditionally replicative adenoviral vectors.

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