Review

Advances in viral-vector systemic cytokine gene therapy against cancer

Lihua Liua, Shijie Wanga, Baoen Shana,⁎, Meixiang Sanga, Shuang Liub, Guiying Wanga

a Research Center, the Fourth Clinical Hospital of Hebei Medical University and Hebei Cancer Institute, 12 Jiankanglu, Shijiazhuang, 050011, China
b Department of Pathology, the Fourth Clinical Hospital of Hebei Medical University, Shijiazhuang, China

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ABSTRACT

Current strategies for cancer gene therapy consist mainly of direct inhibition of tumor cell growth and activation of systemic host defense mechanisms. Cytokine gene-transduced tumor cells have been used as vaccines in clinical trials, which have shown good safety profiles and some local responses but substantial lack of systemic efficacy. Cytokines should be directed at the level of gene selection and delivery, in order to identify the optimal cytokine and achieve efficient and durable cytokine expression at the level of improving immune stimulation. In this review, we will summarize the current achievements of cytokine gene therapy, especially viral-vector, and their applications in cancer treatment. Additionally, we will also discuss and propose future perspectives about cancer gene therapy.

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1. Introduction

In spite of major advances in the comprehension of oncogenesis and treatment of cancer, malignant tumors remain a leading cause of morbidity and mortality worldwide. For centuries, physicians have been fighting cancer using a broad variety of approaches. For solid tumors, radical surgical resection, chemotherapy and radiotherapy are mainstream option routinely combined for the optimal treatment of cancer patients, but unfortunately still fail to cure ~50% of the cases. Cancer gene therapy looks back over one century of clinical applications. This review will summarize the clinical applications of viral-vector systemic cytokine gene therapy to fight human malignancies, focused on the powerful and promising approach of recombinant virus-based cancer immunotherapy.

2. Cytokine gene transfer for cancer therapy in animal studies

Cytokines have pleiotropic effects and mediate systemic and local biological actions, which affect cell growth and differentiation, immune function and inflammation. Systemic administrations of some cytokines such as interleukin-2 (IL-2)[1,2], interferon-α (IFN-α) [3] and IL-12 [4] have resulted in clinical responses in several types of cancer. However, several toxicities limit them in clinical applications. Therefore, studies have looked for more effective and less toxic cytokines and novel routes of administration. Cytokine gene therapy is one of the approaches of immunotherapy, in which cytokine genes in vectors are transduced into cellular vehicles such as tumor cells, dendritic cells (DCs) or fibroblasts. These cellular vehicles express cytokines locally and induce immune responses against the tumor, overcoming the drawbacks of systemic administration of cytokine [5].

Historically, there have been three different approaches applied to gene delivery. The first approach consists of the use of naked
DNA. Direct injection of free DNA to the tumor site has been shown to produce high levels of gene expression and the simplicity of this approach led to its use in a number of experimental protocols [6]. This strategy appears to be limited to tissues that are easily accessible by direct injection such as the skin and muscles and is unsuitable for systemic delivery due to the presence of serum nuclease. The second approach involves using genetically altered viruses. Viral vectors are biological systems derived from naturally evolved viruses capable of transferring their genetic materials into the host cells. Many viruses including the retrovirus, adenovirus, herpes simplex virus (HSV) and adeno-associated virus (AAV) have been modified to eliminate their toxicity and maintain their high capacity for gene transfer hence presenting various advantages [7]. Viral vectors are very effective in achieving high efficiency for both gene delivery and expression. However, the limitations associated with viral vectors, in terms of safety, immunogenicity, low transgene size and high cost, have encouraged researchers to focus on alternative systems. The third approach for delivery systems concerns non-viral vectors, which are mainly of a cationic nature: cationic polymers and cationic lipids. They interact with negatively charged DNA through electrostatic interactions leading to polyplexes and lipoplexes, respectively. The advantages associated with these kinds of vectors include their large-scale manufacture, their low immunogenic response, the possibility of selected modifications and the capacity to carry large inserts (52 kb) [8]. While the transfection efficiency of non-viral vectors is still lower than that for their viral counterparts, a number of adjustments (e.g. ligand attachment) could improve this category of carriers which are, thus far, believed to be the most promising of gene delivery systems. Nonetheless, this class of vectors has to be modified to make systemic delivery possible. To date, systemic administration has resulted in a toxic response (linked to their positive charge), incompatible with clinical applications.

Viral vectors were developed from pathogenic viruses but have been modified by eliminating the functions necessary for virus life cycle in order to reduce pathogenicity and increase safety. These vectors can be used in principle either to introduce the transgene in vitro (ex vivo gene therapy) or can be directly administrated to patients (in vivo gene therapy). Many pre-clinical studies have shown reduction in the tumor growth of established tumors, following intra-tumoral injection of viral vectors or transduced cells carrying cytokines.

2.1. Adenovirus vector

Adenoviruses are double-stranded DNA viruses that can infect both dividing and non-dividing cells [9,10]. The wild type viruses can cause benign respiratory infections in humans. The defective competent adenoviral vectors were first generated by substituting the viral E1 gene with a therapeutic gene. More efficient gene carriers in terms of their capacity [13]. Transfection with adenoviruses coding sequence of the viral genome resulted in better gene carriage and expression. However, the limitations associated with viral vectors, in terms of safety, immunogenicity, low transgene size and high cost, have encouraged researchers to focus on alternative systems. The third approach for delivery systems concerns non-viral vectors, which are mainly of a cationic nature: cationic polymers and cationic lipids. They interact with negatively charged DNA through electrostatic interactions leading to polyplexes and lipoplexes, respectively. The advantages associated with these kinds of vectors include their large-scale manufacture, their low immunogenic response, the possibility of selected modifications and the capacity to carry large inserts (52 kb) [8]. While the transfection efficiency of non-viral vectors is still lower than that for their viral counterparts, a number of adjustments (e.g. ligand attachment) could improve this category of carriers which are, thus far, believed to be the most promising of gene delivery systems. Nonetheless, this class of vectors has to be modified to make systemic delivery possible. To date, systemic administration has resulted in a toxic response (linked to their positive charge), incompatible with clinical applications.

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Adenoviral vectors have been widely used for cancer therapy applications. An example of the deletion-type Ad is ONXY-015, which lacks the E1B-55 kDa-encoding molecule, and the Ad were initially regarded to replicate preferentially in tumors bearing loss of p53 functions [14,15]. Subsequent studies however showed that the Ad replication and the consequent cytotoxicity were not specific to tumor cells or directly related to the status of the p53 gene [14]. Recent reports explained the mechanism of the selective cytotoxic activities to tumors as the enhanced export of viral RNA, which occurred only in tumors [15,16]. Combinatory use of chemotherapeutic agents with ONXY-015 to enhance the anti-tumor effects has been also tested [17,18]. Recent studies on esophageal carcinoma confirmed that the p21(WAF1) correlate with replication of oncolytic viruses for ONXY-015 [19].

To improve the utility of Ad-based gene transfer vectors in the context of clinically applicable cancer gene therapy, it is essential to increase targetting to tumor cells and/or decrease targetting to the liver. Midkine (MK) is a heparin-binding protein expressed in embryonic brain, with multiple biological functions [20,21]. The expression is downregulated in adult normal tissues but the expression was elevated in a number of human tumors including gastrointestinal tumors, breast cancer and lung cancer. A 600-bp region upstream of the initiation site conferred tumor-specific expression of a linked reporter gene and the MK promoter region could activate exogenous gene(s) preferentially in tumors. Tumors but not normal fibroblasts, both of which were transduced with a suicide gene linked with the MK promoter, became susceptible to a prodrug. Studies [22] constructed Ad type 5 (Ad5) in which the MK promoter activated the E1A expression (AdMK). AdMK induced cell death of the transformed cells at a lower multiplicity of infection than that of the normal parental cells. The cytotoxicity of Ad5MK was also greater in a number of tumors than in several kinds of normal fibroblasts. Also the viral propagation of AdMK in tumor cells was greater than that in normal fibroblasts. These data confirmed the oncolytic activity of AdMK in vitro. Recently, the MK gene is found to be expressed in endothelial cells and consequently AdMK is cytotoxic to tumors also by destroying blood vessels.

Ad fiber pseudotyping means alteration of the virus tropism by substituting the receptor-binding proteins (knob/fiber) with those from other serotypes. One such approach is the substitution of Ad5 with a knob from Ad serotype 3 (Ad3). Ad5/3 chimerae have displayed enhanced infectivity in ovarian cancer cells without increasing gene delivery to murine livers [23]. Furthermore, Ad5/3 biodistribution, liver toxicity and rate of blood clearance appear to be no different from Ad5 when administered intravenously or intraperitoneally, suggesting an excellent safety profile for Ad5/3 in preclinical tests. Another fiber-knob alternative is Ad5 bearing the Ad35 fiber-knob portions (Ad5/35), which could produce greater anti-tumor effects than conventional Ad5-based oncolytic viruses. Tumor masses themselves also contain many sorts of normal cells that constitute stroma tissues including fibroblasts and microvesels. These non-tumorous tissues in tumor masses can decrease the cytotoxicity by oncolytic Ad-mediated in vivo because they are derived from many cell lineages and devoid of a strong proliferation activity. One of the strategies to circumvent the “barrier-effects” is to develop oncolytic Ad that are also cytotoxic to the stroma. Ad5/35 is a better vector system than Ad5 in this point, increasing the infectivity to stroma cells. Ad5/35 had greater transduction capability than Ad5 to fibroblasts. Mesenchymal stem cells (MSC) could migrate into tumorous tissues and might contribute to formation of stroma components [24], suggested that oncolytic Ad, which can propagate in such stroma cells by use of an appropriate promoter, can produce greater anti-tumor effects in vivo. Cell growth-related transcriptional activity as shown in MK and survivin promoters could be useful to construct oncolytic Ad since stroma cells proliferate according to the tumor growth. Injection of MSC infected with oncolytic Ad could be one of the therapeutic strategies to overcome the “barrier-effects”. Recent studies demonstrated that Ad5/3 in combination with DC-activating adjuvants may be less likely to induce unwanted side effects such as immune tolerance through the infection of nonprofessional antigen-presenting cells [25]. This indicates a promising therapeutic tool for the in vivo transduction of mature DC by Ad5/3.
2.2. Retrovirus vector

Retroviruses are small RNA viruses with DNA intermediate, which integrate into the host genome producing the viral proteins (gag, pol and env), which are removed when developing the gene delivery carrier. The ability of retroviral vectors to successfully deliver foreign genetic materials was first described in 1981 [26,27].

In a recent study, a retroviral vector was encapsulated with genetic segment bearing both IL-12 family cytokines (IL-12, IL-23 and IL-27) and herpes simplex virus thymidine kinase (HSV-tk) genes [28–30]. While the former provokes anti-tumor immune response, the latter is a suicide gene that activates the produg ganciclovir (GCV) [31]. It is important to note that multiple gene delivery via retroviral vectors is rarely applied due to their limited encapsulation capacity. Most of retroviruses, however, infect actively dividing cells during mitosis [32]. Despite the fact that this feature might protect the normal tissues and provide natural targeting to the tumor, all tumors contain non-dividing cells in the resting phase G0. Such cells can escape the therapy. Lentiviruses such as human immunodeficiency virus (HIV) and their vectors can, however, infect non-proliferating cells [33]. Transfection efficiency was 10 times higher in ovarian cancer cells when lentiviruses were used than when retroviral vectors were used [34]. Tumor regression was observed in more than 40% of treated mice that intratumor injected with lentivirus expressing the HIV-1 vpr gene. [35], capable of cell cycle arrest induction. The usage of lentiviruses, however, has a major drawback because of the original serious clinical consequences of these viruses. In this context, new retroviral vectors namely replication-competent retroviruses were developed and engineered to replicate specifically in the targeted neoplastic tissues; thus, increasing the vectors’ transduction non-toxic ability [36].

3. Results of clinical trials

Following results of pre-clinical experiments, many clinical trials have been conducted, based on irradiated autologous or allogeneic tumor cells as vehicles for cytokine delivery. Due to the knowledge of specific melanoma-associated antigen (including gp100, tyrosinase and MAGE, GAGE, BAGE family genes), many studies focused on melanoma. Autologous melanoma cells were established from surgical specimens, engineered for the production of various cytokines, including IFN-γ, IL-2 or GM-CSF, and injected intra-tumorally. Although the procedure was well tolerated, however, specific anti-tumor CTLs and local inflammatory infiltration were observed only in some patients [37–39]. Similar studies were extended to patients with prostate and renal cancer, and anti-cancer immune responses were detected [40,41].

In order to allow a constant and well-characterized transgene expression, allogeneic tumor cell lines were necessary to exploit. Allogeneic cells, encoding for IL-2 or IL-4, were used for the treatment of melanoma, with minor clinical responses [42,43]. Recently, Maio et al. [44] demonstrated that repeated immunization with allogeneic IL-4–producing melanoma cells generated antibodies directed against vaccinating and autologous melanoma cells in over 60% of the patients, thus suggesting break of tolerance against the autologous tumor.

Xenogenic cytokine-producing fibroblasts have also been injected into solid tumors (melanoma, breast and colon cancer), with the goal of inducing immune responses against the xeno-antigens which could cross-react and eliminate tumor cells. Intriguingly, the treatment induced tumor regression in about 50% of patients, due to a local inflammatory response and, likely, NK-mediated mechanisms; however, tumor-specific CTLs were not generated, which may explain the lack of regression at untreated sites [45,46].

The next wave of clinical trials has seen the advent of viral vectors as cytokine delivery tools. A few clinical trials have foreseen the intra-tumoral injection of retroviral vectors encoding IFN-γ in melanoma patients: clinical results have been rather disappointing, however, conceivably due to some intrinsic limitations of these vectors including their short half-life of and the requirement for proliferation cells as suitable targets [47].

Driven by the promising results obtained in animal studies, recombinant Ad vectors, which can be produced at very high titers under GMP conditions and infect both proliferating and resting cancer cells with adequate efficiency [48], have been used in most clinical studies. Ad-mediated IL-2 gene transfer was used in several phase I clinical trials for lung [49], prostatic [50], breast cancer and melanoma [51]. Treatment induced a local inflammatory response, mediated by CD3+CD8+ T lymphocytes, associated to tumor necrosis, but only transient and local tumor responses. Recent study demonstrated that IL-12 was administered to patients after first line chemotherapy for ovarian/peritoneal carcinoma in clinical phase II study, with better results [52].

Another novel clinical gene therapy approach followed the introduction of TNFerade, a second-generation Ad carrying the human TNF-α driven by the early growth response 1 (Egr-1) promoter [53]. The Egr-1 promoter is activated by both radiation and different classes of chemotherapeutic agents that boost the radical oxygen intermediate (ROI) production (such as cisplatin, 5-fluourouracil, doxorubicin and paclitaxel) [54]; thus, TNF-α release can be hypothetically controlled in a time and spatially regulated fashion. So far, tumor-targeted Ad for systemic treatments are not available; thus treatment of tumors by radio- and chemo-induction is presently limited to accessible sites of local diseases. Phase I clinical trials performed in patients with solid tumors (pancreatic, head and neck, hepatocellular, breast, colorectal cancer and melanoma) [55] and soft-tissue sarcomas [56] confirmed that TNFerade and radiation are well tolerated and that this treatment can be effective for established tumors; in fact, complete tumor regression has been occasionally observed. Histopathological analysis of tumor samples treated with the combination of TNFerade and radiation revealed extensive intra-tumoral vessel thrombosis and tumor necrosis, whereas adjacent normal tissue and vessels remained apparently unaffected [57,58]. Thus, the treatment exerted both cytotoxicity effects on both tumor cells and the tumor microvasculature. Several phase II trials were subsequently started in patients with pancreatic adenocarcinoma and adenocarcinoma of the esophagus in combination with 5-fluorouracil and cisplatin chemotherapy. The trial in esophageal cancer patients was put on hold due to an increase in serious thromboembolic events. Trials for patients with rectal cancer, metastatic melanoma and soft-tissue sarcoma are also being initiated [59].

Although a phase III study with ONYX-015 for head and neck cancer (Onyx Pharmaceuticals) was prematurely halted in the States, a Shanghai-based company, Shanghai Sunway Biotech, recently acquired the Chinese FDA approval of H101 (Oncocrine), which is functionally identical to ONYX-015 [60]. Their clinical outcomes obtained with Chinese patients, combination of H101 with cisplatin for head and neck cancer, are reported to be similar to those with Onyx Pharmaceuticals. These encouraging data, although precise comparative analyses are probably required, hopefully boosts clinical application of oncolytic virus therapy not only in China but also in other countries.

4. Conclusion

Cytokine-based gene therapy has already entered its most critical phase: it has been administered to cancer patients. Although
therapeutic results have not been impressive, clinical trials have clearly documented the feasibility and safety of this approach. It is therefore quite possible that cytokine gene delivery will become a routinely available form of cancer therapy in near future.

Finally, it seems clear that the expression of a single transgene is unlikely to be sufficient to eradicate a tumor, in particular when it is diagnosed late in disease progression. Hence, modularity therapy, including conventional therapy (surgery, chemotherapy and radiotherapy) with one or more transgenes will have to be considered to provide a “chance” of success.

References


