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Concise Review

Gene therapy of liver cancer

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Abstract

Gene therapy of liver cancer covers a variety of gene transfer strategies aimed to the treatment of patients with primary and secondary liver tumors, including gene directed enzyme/pro-drug therapy, inhibition of oncogenes and restoration of tumor-suppressor genes, immunotherapy, anti-angiogenesis and virotherapy. Some of these strategies have reached early clinical development with diverse little success.

Key words: Gene therapy, cancer, liver, hepatocellular carcinoma.

Introduction

Gene therapy consists in the transfer of genetic material to a patient with the aim to correct a disease. Gene delivery can be performed directly into the subject, using a variety of vehicles named vectors (*in vivo* gene therapy), or it can be done on isolated cells *in vitro* that are subsequently introduced into the organism (*ex vivo* gene therapy).

Cancer has been the main focus of gene therapy approaches[1] for several reasons. First, the genetic alterations that contribute to the malignant transformation of cells are being unravelled with increasing detail in the last two decades, and this provides multiple candidate targets for gene therapy intervention.[2] And second, the dismal prognosis of most patients with advanced cancers results in a desperate need for new therapeutic interventions and influences the risk-benefit balance that is key to clinical development of such a new platform.

Liver cancer is a good example of this situation. Hepatocellular carcinoma (HCC) accounts for 80% of primary liver tumors in adults, it has an increasing incidence[3] and a poor 5-year survival rate of about 7% despite treatment.[4] In addition, the liver is the most frequent site of metastasis, especially from gastrointestinal cancer. Potentially curative therapies such as liver transplantation and surgical resection can only be applied to a minority of subjects because of the advanced disease at the time of diagnosis and the lack of suitable organ donors. Other regional treatments may be beneficial for unresectable HCC, but local failure or recurrence are frequent and long term survival remains poor. In this context, gene therapy could be considered as a potential adjuvant of other therapies. Clinical trials performed so far have shown that side effects are acceptable in most of the cases, and the mechanism of action is different from standard treatments.[5] Therefore, choosing the right combination among gene therapy approaches and conventional treatments may achieve a synergistic effect. Furthermore, the refinement of interventional therapies for HCC provides new possibilities for the delivery of gene therapy vectors into hepatic tumors, increasing the effective dose and minimizing potential side effects derived from non-target cell transduction.

Restoration of tumor suppressor genes

This strategy is the most intuitive application of gene therapy for the treatment of HCC and other cancers. It is clear that the loss of function of certain genes is associated with malignant transformation of cells. Under experimental conditions, it has been shown that restoration of tumor suppressor genes can revert the malignant cell phenotype. However, therapeutic application of this observation faces enormous difficulties. Cancer cells often suffer some degree of genetic instability. When they lose their capacity to sense and repair damaged genes, mutations accumulate and cells with higher proliferation rate and lower sensitivity to apoptotic stimuli are selected sequentially. Under these circumstances, they may become insensitive to the restoration of a particular tumor suppressor gene. On the other hand, this approach would require the introduction of the gene and the expression of the antitumoral protein virtually in all cancer cells, or at least in those responsible for the tumor maintenance. This is technically impossible with current gene therapy vectors, especially for solid tumors like HCC.

Despite all these considerations, the transfer of p53 tumor suppressor gene has shown effect in several animal models of cancer, including HCC.[6,7] This proof of con-

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cept has stimulated the use of p53 as a therapeutic gene. Mutations in p53 or alterations in its pathway have been described in more than 50% human cancers. When cells lack functional p53, they accumulate mutations that led to malignant initiation, progression and resistance to treatments. Thus, the restoration of p53 may render tumor cells sensitive to apoptotic stimuli, even if they have accumulated other mutations. This may explain the therapeutic effect observed in pre-clinical models, and suggests a potential role of p53 as an adjuvant for conventional therapies that induce apoptosis in cancer cells.

In contrast, several clinical trials based on delivery of the wild type p53 gene using different vectors have observed variable, often less positive results in different types of cancer such as lung, head and neck, bladder, ovarian and breast cancer.[8] However, a first-generation adenoviral vector expressing p53 became the world's first commercially licensed gene therapy product (Gendicine) for the treatment of head and neck squamous cell carcinoma in China.[9] In a clinical trial performed on 30 HCC patients, partial response (PR) was reported in 2 cases. In another HCC clinical trial, Gendicine in combination with transarterial chemoembolization (TACE) reportedly achieved a 67% PR rate. The clinical significance of these results is controversial at this time, but the availability of a gene-based therapy in the market with potential effect on HCC will probably extend its use in combination with other therapies and allow the identification of synergistic effects.

Inhibition of oncogenes

Correction of the imbalance between positive and negative proliferation signals can be attempted by inhibiting the function of genes implicated in the maintenance of unrestricted cell proliferation and acquisition of metastatic phenotype. Many of the drawbacks mentioned above can be applied here, like the need of a highly efficient gene transfer method and a dominant role of the target gene in malignant transformation. The number of candidate oncogenes is continuously expanding as the knowledge of cancer at the genomic and proteomic levels advances. Hopefully, the inhibition of oncogene expression will not only decrease cell proliferation, but also restore sensitivity of cells to apoptotic stimuli. For instance, it is known that the inhibition of the Ras oncogene, apart from blocking a cascade of mitotic signals, relieves the repression exerted on the p53 pathway and predisposes cells to apoptosis.[10] This may be the case for other oncogenes such as the pituitary tumor transforming gene 1 (PTTG1).[11] Another example is the catalytic subunit of telomerase (telomerase reverse transcriptase, TERT). Since telomerase function is necessary to maintain the telomere length in each cell division, cancer cells undergoing unrestricted cell proliferation present activation of TERT expression. Therefore, inhibition of TERT was supposed to cause inhibition of cell growth after several divisions, when telomeric repeats finally run out. However, efficient inhibition of telomerase expression is able to induce apoptosis in a few days.[12]

Different methods are used to inhibit expression of oncogenes. One of them is based on the transfer of antisense nucleotides, artificial sequences complementary to the mRNA corresponding to the gene whose inhibition is attempted.[13] These can be short sequences (antisense oligonucleotides, ASO), or the full cDNA. Several mechanisms account for the blocking of gene expression, with the most widely spread and studied being the degradation of RNA-DNA hybrids by cell nucleases. A more recent approach is RNA interference, another posttranscriptional gene silencing mechanism based on the production of double-stranded stretches of RNA complementary to the target mRNA.[14] Using the endogenous cell machinery, the double-stranded RNA is processed into short interfering RNAs (siRNAs) that recognize the cognate mRNA and trigger its degradation. Alternatively, the siRNAs can be transfected directly. In the "triple helix" strategy, the inhibitory oligonucleotides (triplex-forming oligonucleotides, TFOs) are targeted to the cellular double-stranded DNA.[15] They interact with polypurine-polypyrimidine sequences in the minor or major grove of genomic DNA and block gene expression at different levels depending on the localization of the complementary sequence. They could be potentially used not only for gene expression modification, but also in gene correction strategies.[16] Finally, the expression of secreted or intracellular antibody-based molecules has been proposed to block the function of oncogenes.[17,18]

In the case of HCC, the inhibition of several genes has shown potential antitumor effect. Most reports provide proof of concept showing growth inhibition or induction of apoptosis using HCC-derived cell lines in cell culture. Studies in animal models show growth retardation in tumors, especially when cancer cells are transfected *ex vivo*, but complete eradication is difficult when in vivo gene therapy is tested on pre-existing tumors. Since telomerase and Wnt pathway activation are frequently associated with HCC, different approaches including antisense molecules and siRNA have been used to inhibit them.[19-21] Antisense technology was also used against FGF-2,[22] VEGF (23) and COX-2 genes.[24] The triplex helix approach showed similar results as antisense technology for the inhibition of IGF-I and induction of apoptosis in HCC cells.[16] The inhibition of PTTG1 and urokinase-type plasminogen activator (u-PA) has been accomplished using siRNA on HCC cells.[25] The p28-GANK oncoprotein, which induces hyperphosphorylation and increased degradation of pRB was found overexpressed in the majority of HCCs, and repeated administration of an adenoviral vector that induces the production of siRNA against p28-GANK caused a dramatic decrease in the growth of human HCC xenografts in nude mice.[26] This shows that the

continuous inhibition of an oncogene may have a strong impact on the progression of tumors. The clinical application of this approach is challenging, because highly efficient long-term expression vectors will be needed instead of first generation adenoviruses.

Gene-directed enzyme/pro-drug therapy (GDEPT)

This approach is based on the transfer of exogenous genes that convert a non-toxic pro-drug into a cytotoxic metabolite in cancer cells. Once the pro-drug is administered systemically, transduced cells expressing the converting enzyme die and, in some cases, provoke the destruction of surrounding cells (bystander effect). Unlike other gene therapy strategies, GDEPT lacks intrinsic tumor specificity, and relies on tumor targeting at the levels of cell transfer (depending on the vectors and the route of administration) and gene expression (depending on tumor-specific promoters).[27] The efficacy of a GDEPT system is highly influenced by the extent of the bystander effect, because the fraction of transduced cells in a tumor is generally low with current gene therapy vectors.[28]

The thymidine kinase gene from HSV-1 (HSV-TK) used in conjunction with the pro-drug ganciclovir (GCV) was the earliest and most used GDEPT system applied to HCC and other cancers.[29] It has shown significant antitumor effect in relevant animal models of HCC, such as carcinogen-induced HCC in rats.[30] HSV-TK converts ganciclovir into the monophosphate intermediate that is subsequently transformed into the triphosphate form by cellular enzymes. Ganciclovir-triphosphate is incorporated into the DNA and causes apoptosis in a cell cycle-dependent manner, but it can cause mitochondrial toxicity in normal hepatocytes if the expression of HSV-TK is not restricted to HCC cells.[31,32] Apart from the therapeutic purpose, HSV-TK can be considered a reporter gene for PET analysis. It has been successfully used to visualize transduction of HCC with adenoviral vectors in humans.[33] So far, the good antitumor efficacy of the HSV-TK system observed in different animal models of HCC has not been demonstrated in the clinical setting.[29]

The yeast Cytosine Deaminase converts the antifungal drug 5-fluorocytosine (5-FC) into the cytotoxic thymidylate synthetase inhibitor 5-fluorouracil (5-FU).[34] This metabolite can diffuse locally and cause wider bystander effect than phosphorylated ganciclovir, but the cytotoxicity is also cell cycle-dependent. The system has been used in animal models of primary and metastasic liver cancer with good results.[35,36] The efficacy of 5-FU on HCC patients is very low, but this strategy could achieve high local concentrations of the drug. In this context, toxicity on normal liver should be carefully evaluated. In addition, the conversion of 5-FC to 5-FU by the cytosine deaminase present in habitual enterobacteria can contribute to toxicity.[37]

Other GDEPT approaches generate very potent DNA cross-linking agents whose effects are largely cell cycle-independent. These include the cytochrome P450/cyclophosphamide[38] and the Nitroreductase/dinitrobenzamide CB systems. Regarding the latter,[39] intratumor administration of a first generation adenoviral vector expressing Nitroreductase in HCC patients is safe and feasible. Transgene expression was dose-dependent and is supposed to be clinically relevant, although no pro-drug was administered to patients in this study. Strong immune responses against the vector and the therapeutic gene were observed, indicating that re-administration of the treatment may not be beneficial. The antitumor effect and toxicity of this approach in patients receiving the pro-drug requires new clinical trials.

An approach closely related to GDEPT consists on the delivery of the sodium iodide symporter (NIS) gene to cancer cells.[40] Since NIS is necessary for the internalization of ^{131}I in the cell, a higher dose would be accumulated in cells expressing NIS, as it happens in thyrocytes, resulting in cell cycle blockade and death. Using this method, the extent and location of gene transfer can be detected by tomography. An adenovirus vector expressing NIS under the control of the CMV promoter has been used for the treatment of HCC in a model of chemically induced tumors in rats.[41] After injection of the vector in pre-existing nodules, specific accumulation of ^{131}I and significant reduction in tumor volume was observed.

Targeted expression of cytotoxic/pro-apoptotic genes

This strategy is based on the selective transfer of genes that will cause the destruction of the cancer cells by different mechanisms. The concept is similar to GDEPT, but in this case the effect does not depend on any exogenous drugs. This can be an advantage in same circumstances, but on the other hand it lacks the possibility of modulating the cytotoxicity pharmacologically. This means that the system relies mostly on the targeting of gene transfer and expression into cancer cells, using specific surface ligands or promoters. The promoters for alpha-fetoprotein (AFP) and TERT have been used to control the expression or the diphtheria toxin fragment A and other cytotoxic genes in HCC cells,[42,43] but the toxicity of these treatments on relevant animal models is unclear. Alternatively, the mechanism of action of the lethal gene can provide some tumor specificity. This is the case of TNF-related apoptosis inducing ligand (TRAIL). Unlike other members of the TNF ligand family, such as FASL and TNF-alpha, TRAIL induces apoptosis preferentially on cancer cells and may have reduced heptotoxicity.[44] The extracellular domain of TRAIL works as a soluble cytokine (sTRAIL) and induces apoptosis on cancer cells at distant locations from the producing cell. In fact, an AAV vector expressing sTRAIL fused with a

human insulin signal peptide has shown potent antitumor effect on subcutaneous liver cancer xenografts after oral or intraperitoneal administration of the vector.[45] This systemic effect was achieved without significant liver toxicity. Other vectors developed for the expression of TRAIL include first generation and oncolytic adenoviruses with enhanced infectivity on cancer cells.[46,47]

Genetic immunotherapy

The transfer of genes with the aim to elicit an immune response against tumors is one of the most extensively used strategies in the field of cancer gene therapy. It is based on the observation that cancer cells modify their [illegible] in order to avoid being detected and rejected. If this can be reversed, the specificity and memory nature of the immune system offers the possibility of controlling the primary tumor and metastasis, which is the ultimate goal of all oncologic treatments. The wide repertoire of immunogene therapy approaches can be grouped as follows:

[illegible]

Cytokines are key mediators in the function of the immune system. They have been extensively used to stimulate the immune response against tumors, including interleukins 2, 7, 12, 15, 18, 21, 23 and 24; interferon alpha, beta and gamma; tumor necrosis factor alpha; granulocyte-macrophage colony stimulating factor (GM-CSF), and others. Their effect on different cell components of the immune system and their influence on the expression of endogenous factors are extremely complex. Most of these cytokines do not have an intrinsic tumor-specific effect, but they may enhance the precarious immune response against tumors if the dose, location and timing are carefully controlled. For example, interleukin-12 (IL12) promotes a T-helper cell type 1 (Th1) response with activation of cytotoxic T lymphocytes and natural killer cells (NK), together with an antiangiogenic effect. These effects are largely dependent on the induction of IFN-gamma. The systemic administration of recombinant IL12 showed potential antitumor effects in humans, but severe toxicity was observed and this modality of treatment was discarded. The use of gene therapy vectors enables the localization of IL12 expression in the tumor, especially if vectors with liver tropism such as those derived from adenovirus are used.[48] The antitumor effect of this strategy on different animal models of HCC has been demonstrated by several groups.[49,50] Tumor eradication and immunologic protection against relapse is achieved in a significant proportion of cases, including implanted tumors in syngenic animals and chemically-induced HCC in rats. These results led to a phase I clinical trial that demonstrated the safety and feasibility of intratumor injection of a first generation adenoviral vector expressing IL12 in primary and metastatic liver cancer patients.[51] Using these vectors, the expression of IL12 was very low and transient. No complete responses were observed, but patients with HCC had a better outcome than other histological groups in this trial. Based on these results, improvements in the vectors are being investigated. The use of high-capacity adenoviral vectors carrying a liver-specific inducible system for the expression of IL12 allows the long-term expression of the cytokine in response to the inducer mifepristone. Using this vector, the levels and duration of cytokine expression can be modulated to achieve antitumor effect and avoid toxicity.[52] Further improvement can be achieved by using a version of IL12 in which the p35 and p40 subunits are fused in a single protein using a short linker peptide.[53] Experiments performed in rats bearing HCC indicate that the single chain IL12 is about 1000 times more potent than the native protein when an equivalent adenoviral vector is used to deliver the gene intratumorally.[50]

Other cytokines that deserve special attention are TNF-alpha and IL24, that have shown antitumor effect on animal models of HCC,[54] and ongoing clinical trials suggest the potential therapeutic effects on other malignancies in humans.[55] IL24 is especially promising, because apart from its immune-regulatory activities it induces apoptosis preferentially in cancer cells.

Taking into account the natural mechanism of immune response activation, pro-inflammatory cytokines and co-stimulatory signals should be combined to achieve an effective response and avoid anergy. It is possible that the accessory signals are already present in the tumor, but there is evidence of enhanced antitumor effect when IL12 is transferred together with 41BB agonists[56] or B7.1[57] in animal models of HCC. The intratumoral injection of an adenoviral vector expressing CD40L achieved tumor eradication on a significant proportion of pre-existing HCC in a rat model.[58] This molecule is normally expressed on activated T cells and interacts with CD40 on the surface of antigen-presenting cells.

A combination of different cytokines may be more effective and less toxic than the expression of a single cytokine at high levels. The injection of adenoviral vectors expressing IL12 and IP-10 (interferon-gamma inducible protein-1) exerted a synergistic antitumor effect in a murine model of colon cancer when both molecules were expressed locally.[59] This is in agreement with the "attraction and activation hypothesis", in which colocalization of immunostimulatory (IL12) and chemoattractant factors (IP10) is needed. Some pre-clinical data indicate that IL15 can increase the antitumor effect IL12 on HCC models.[60] Other combinations proposed for the treatment of HCC include IL12+GM-CSF[61] or IL12+MIP3 alpha.[62] Finally, the antitumor effect of cytokines can be enhanced by other gene therapy approaches like GDEPT using HSV-TK, as demonstrated by several groups that employed adenoviral or retroviral vectors for gene delivery in HCC models.[63,64]

Vaccination with tumor antigens and genetically modified cells

The transfer of genes encoding tumor-specific antigens such as AFP has been used with the aim to break the immune tolerance against HCC.[65] The pre-clinical efficacy of this approach depends on the particular animal model employed,[66] suggesting that high variability could be expected in patients. A different approach consists on the administration of activated effector or antigen-loaded presenting cells to fight cancer. The efficacy of these cells can be increased if they are manipulated genetically to express antigens, cytokines or co-stimulatory molecules (*ex vivo* gene therapy). Syngeneic fibroblasts or cancer cells expressing IL12[67] or IL2 plus B7[68] can trigger an immune response against HCC in murine models. However, the use of cancer cells as a source of antigens and cytokines poses obvious technical difficulties in the clinical setting. An attractive alternative is the use of autologous dendritic cells (DC), professional antigen presenting cells that express the co-stimulatory molecules (CD80, MHC class I and II, etc.) necessary for efficient activation of effector cells. DCs expressing AFP,[69] cytokines[70] or co-stimulatory molecules[71] have been successfully used in animal models of HCC and gastrointestinal cancer.[72] These results encouraged the initiation of a phase I clinical trial in which DCs expressing IL12 after ex-vivo infection with an adenoviral vector were injected into the tumor mass.[73] However, it was demonstrated that the cells were unable to migrate to lymph nodes because they were sequestered into the tumor by local factors,[74] preventing an efficient activation of effector cells and the establishment of relevant antitumor immnune responses.

Adoptive cell therapy consists on the infusion of autologous T cells or killer cells that have been expanded and activated *in vitro*. In animal models, it has been demonstrated that T cell expansion occurs *in vivo* in tumor-bearing mice that were treated with IL12.[75] The infusion of these cells has antitumor effect on recipient mice, in synergy with in vivo gene therapy by an adenoviral vector expressing IL12. This suggests that gene immunotherapy can be used in combination with adoptive T-cell therapy in order to increase the efficacy observed in clinical trials that used either strategy alone.

Anti-angiogenic gene therapy

Since tumor growth requires intense neo-vascularization, a series of approaches aimed to specifically block the cancer-induced formation of new vessels have been developed. Anti-angiogenic factors such as endostatin have been identified and have demonstrated the ability to inhibit tumor growth *in vivo*. Since HCC is known to be much vascularized, antiangiogenic therapies may have a strong therapeutic benefit, probably in combination with other standard or experimental treatments. Gene therapy may play an important role in this field, because anti-angiogenic factors need to be delivered for long period of times to control the progression of tumors. The combination of endostatin delivered by an AAV vector and chemotherapy (etoposide) achieved antitumor effect on metastatic liver cancer in mice.[76]

Other anti-angiogenic approaches are focused on blocking the VEGF receptor, which is an important mediator of angiogenesis. This can be achieved by expressing the soluble form of VEGF receptor, which sequesters VEGF.[77] The same approach has been used to block the endothelium-specific receptor Tie2, which affects direct tumor growth and neovascularization.[78] The Pigment Epithelium Derived Factor (PEDF) has been recently discovered as an anti-angiogenic protein expressed in normal liver[79] that is downregulated in HCC patients, suggesting a possible role in tumor progression. The transfer of PEDF has antitumor effects in a murine model of HCC.[80] NK4 is a fragment of the Hepatocyte Growth Factor (HGF) that acts as a HGF antagonist and blocks angiogenesis. The intrasplenic administration of an adenoviral vector expressing a secreted form of NK4 caused reduction in the vascularization and growth of pancreatic metastasis in the liver of mice.[81] Finally, it should be mentioned that the inhibition of angiogenesis may be one of the most important mechanisms by which IL12 exerts its antitumor effect.[82]

Oncolytic viruses

Using the cytopatic effect of certain viruses to destroy cancer cells is an old idea, but the advances in viral vector design and production have renewed the interest in the field of virotherapy. The objective is to obtain a virus that replicates and kills preferentially cancer cells, leaving the surrounding normal tissues relatively intact.[83] This property is intrinsic of some viruses. For instance, Vesicular Stomatitis Virus (VSV), Measles Virus (MV) and Newcastle Disease Virus (NDV) are very sensitive to the inhibitory effects of IFN and replicate only in cancer cells that have developed mechanisms to counteract IFN pathways. Other viruses like reovirus replicate better in cells that present activation of the Ras oncogene.[84]

On the other hand, other viruses such as Adenovirus or HSV can be genetically modified to make their replication cancer-specific. One of the methods to achieve cancer specificity is the deletion of viral functions necessary for replication in normal cells, but not in cancer cells. For instance, the adenoviral protein E1A blocks pRB in the cell to force activation of the cell cycle, whereas E1B 55K blocks p53 to inhibit apoptosis at early times. Since both p53 and pRB pathways are commonly altered in cancer cells, adenoviruses lacking these functions will replicate preferentially in tumors.[85,86] Another method to restrict the replication of viruses is to use tumor-specific

promoters to control the transcription of viral genes important for replication, such as E1A and E4 for adenovirus.[87] Parallel strategies have been used to achieve oncolytic herpes viruses.[88,89] An important property of oncolytic viruses is the possibility of accommodating therapeutic genes an act as gene therapy vectors with the advantage of tumor-specific amplification of gene expression.[90] These genes code for pro-drug converting enzymes, immunostimulatory cytokines or pro-apoptotic proteins that enhance the oncolysis and/or achieve a systemic effect.

The mutant dl1520 adenovirus (also called ONYX-015) was described in 1996 as the first oncolytic adenovirus. It contains a deletion in the E1B 55K gene that achieves preferential replication in cancer cells by different mechanisms.[91] Although recent advances have yielded viruses with improved potency and specificity, the experience accumulated with ONYX-015 in the laboratory and in the clinic has been extremely useful for the advance of the field. The virus has shown partial antitumor effect on murine models of HCC,[92] and clinical trials for other cancers indicate a potential benefit when used in combination with chemotherapy.[93] In the case of liver cancer, a clinical trial on HCC patients showed no evident antitumor effect.[94] In a separate phase II trial in patients with metastatic colorectal cancer the virus was administered intravenously, and only transient stabilization of the disease could be observed in some cases.[95] When the virus was administered intratumorally in a different clinical trial for hepatobiliary tumors, transient reduction of serum tumor markers was observed in 50% patients, although radiological responses were less than 10%.[96] These results support the notion that ONYX-015 has limited therapeutic effect as monotherapy on HCC patients, especially if systemic routes are used. When the virus was administered intravenously in combination with 5-Fluorouracil and leucovorin in patients with liver metastases of gastrointestinal cancers, 25% cases presented partial or minor (< 50%) radiological responses, with good tolerance and evidence of adenovirus replication in tumors.[98]

Other oncolytic adenoviruses have been developed, and show promising results (usually better than ONYX-015) in animal models of HCC. However, their performance in clinical trials has not been tested so far. The AFP promoter was used to control the expression of the E1A viral gene, with or without E1B 55K deletion, and this achieves preferential replication in AFP-producing HCC cells.[98,99] The same is observed in metastatic gastrointestinal cancer using a virus controlled by the CEA promoter.[100] A broader cancer spectrum is achieved when other tumor-specific promoters such as human TERT[101,102] and E2F-1[103] are used. The efficacy of these agents can be increased if they are adapted as gene therapy vectors for therapeutic genes ("armed" viruses), because viral oncolysis usually cooperates with the effect of the gene. Oncolytic adenoviruses expressing GM-CSF,[104] TRAIL,[105] Smac,[106] Cytosine Deaminase[107] and endostatin[108] have demonstrated better performance than the previous versions.

The field of virotherapy has been enriched by the incorporation of oncolytic agents derived from different viruses, which may solve some of the limitations observed with adenovirus. For instance, HSV-1 exerts a potent oncolytic effect and its large genome can accommodate different exogenous genes, apart from the endogenous TK.[109] The complex genome of HSV-1 allows multiple modifications that can be exploited to achieve tumor specificity. The G207 mutant contains a disruption in the UL39 gene that eliminates the ribonucleotide reductase function and determines preferential replication in cancer cells, and efficient elimination of HCC cells has been reported with this virus.[110] The rRp450 HSV-1 variant carries the cytochrome p450 gene as a pro-drug converting enzyme. This virus has shown promising antitumor effect on HCC models,[111] although complete eradication of metastatic liver cancer was not observed after single or multiple intraportal administrations. In addition, significant antitumor effect has been obtained in liver cancer models using herpes virus expressing Cytosine deaminase[112] or IL12.[113]

Finally, VSV-derived viruses are emerging as a new class of oncolytic agents. A single injection of a recombinant VSV virus into the hepatic artery increased the survival of rats bearing multifocal HCC, and multiple doses achieved long term survival and tumor eradication in nearly 20% of the animals.[114]

Conclusion

Therapy of liver tumors, both primary and metastatic, remains a challenge that needs new approaches. Gene therapy is an experimental discipline in continuous evolution that offers interesting opportunities for the treatment of liver cancer. From the early excitement about gene therapy possibilities, the field soon realized its limitations and is now systematically addressing fundamental issues to solve them. The transfer of genes to the majority of cancer cells is still unrealistic for solid tumors, even with the best vectors available to date. Immunogene therapy approaches try to circumvent this limitation and extend the antitumor effect to distant metastases. Pre-clinical studies have validated the concept, but at the same time the results in animal models reveal that the efficacy of immunotherapy is very limited in advanced liver cancer. Since this is the scenario in which early phase clinical trials are usually being conducted, it is not surprising that the results are apparently deceiving. Oncolytic adenoviruses were envisioned as autonomous therapeutic agents that would seek and destroy cancer cells, amplifying the initial load until the tumor is eradicated.

Now we know that they find important physical barriers that limit their distribution inside the tumor. Moreover, the immune system will control the spread of the viruses in a few days and neutralize further administrations, leaving a narrow time frame for them to display their oncolytic activity. An additional obstacle for the clinical application of most gene therapy approaches is the cost and technical difficulties for the large scale production of the vectors. Despite all these difficulties, gene therapy may play an important role as an adjuvant of other standard or experimental treatments against liver cancer in the near future. There is evidence that different gene therapy approaches like GDEPT or oncolytic viruses have synergistic effects when combined with chemotherapy or radiotherapy. The different mechanisms of action favour these combinations and may prevent the development of resistance to the treatment. As the knowledge of tumor immunology advances, more rational immunogene therapy approaches are designed. In addition, the improvement of invasive techniques for locoregional treatment of HCC can be used to deliver gene therapy vectors inside the tumor, increasing their safety and efficacy. In summary, gene therapy will improve the management of liver cancer patients in the future, probably as part of an individualized multimodal therapy. This will require close collaboration and a continuous flow of information between basic, applied researchers and health care professionals.

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References

1. Edelstein ML, Abedi MR, Wixon J, Edelstein RM. Gene therapy clinical trials worldwide 1989-2004-an overview. *J Gene Med* 2004; 6: 597-602.
2. Lee JS, Thorgeirsson SS. Genome-scale profiling of gene expression in hepatocellular carcinoma: classification, survival prediction, and identification of therapeutic targets. *Gastroenterology* 2004; 127: S51-55.
3. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; 127: S5-S16.
4. Carr BI. Hepatocellular carcinoma: current management and future trends. *Gastroenterology* 2004; 127: S218-224.
5. Prieto J, Qian C, Hernandez-Alcoceba R, Gonzalez-Aseguinolaza G, Mazzolini G, Sangro B, Kramer MG. Gene therapy of liver diseases. *Expert Opin Biol Ther* 2004; 4: 1073-1091.
6. Anderson SC, Johnson DE, Harris MP, Engler H, Hancock W, Huang WM, Wills KN, Gregory RJ, Sutjipto S, Wen SF, Lofgren S, Shepard HM, Maneval DC. p53 gene therapy in a rat model of hepatocellular carcinoma: intra-arterial delivery of a recombinant adenovirus. *Clin Cancer Res* 1998; 4: 1649-1659.
7. Mitry RR, Sarraf CE, Havlik R, Habib NA. Detection of adenovirus and initiation of apoptosis in hepatocellular carcinoma cells after Ad-p53 treatment. *Hepatology* 2000; 31: 885-889.
8. Haupt S, Haupt Y. Manipulation of the tumor suppressor p53 for potentiating cancer therapy. *Semin Cancer Biol* 2004; 14: 244-252.
9. Peng Z. Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. *Hum Gene Ther* 2005; 16: 1016-1027.
10. Halaschek-Wiener J, Wacheck V, Kloog Y, Jansen B. Ras inhibition leads to transcriptional activation of p53 and down-regulation of Mdm2: two mechanisms that cooperatively increase p53 function in colon cancer cells. *Cell Signal* 2004; 16: 1319-1327.
11. Cho-Rok J, Yoo J, Jang YJ, Kim S, Chu IS, Yeom YI, Choi JY, Im DS. Adenovirus-mediated transfer of siRNA against PTTG1 inhibits liver cancer cell growth in vitro and in vivo. *Hepatology* 2006; 43: 1042-1052.
12. Saretzki G, Ludwig A, von Zglinicki T, Runnebaum IB. Ribozyme-mediated telomerase inhibition induces immediate cell loss but not telomere shortening in ovarian cancer cells. *Cancer Gene Ther* 2001; 8: 827-834.
13. Dean NM, Bennett CF. Antisense oligonucleotide-based therapeutics for cancer. *Oncogene* 2003; 22: 9087-9096.
14. Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. *Nature* 2004; 431: 371-378.
15. Trojan LA, Kopinski P, Wei MX, Ly A, Glogowska A, Czarny J, Shevelev A, Przewlocki R, Henin D, Trojan J. IGF-I: from diagnostic to triple-helix gene therapy of solid tumors. *Acta Biochim Pol* 2002; 49: 979-990.
16. Upegui-Gonzalez LC, Ly A, Sierzega M, Jarocki P, Trojan L, Duc HT, Pan Y, Shevelev A, Henin D, Anthony D, Nowak W, Popiela T, Trojan J. IGF-I triple helix strategy in hepatoma treatment. *Hepatogastroenterology* 2001; 48: 660-666.
17. Bilbao G, Contreras JL, Curiel DT. Genetically engineered intracellular single-chain antibodies in gene therapy. *Mol Biotechnol* 2002; 22: 191-211.
18. Nguyen TH, Loux N, Dagher I, Vons C, Carey K, Briand P, Hadchouel M, Franco D, Jouanneau J, Schwall R, Weber A. Improved gene transfer selectivity to hepatocarcinoma cells by retrovirus vector displaying single-chain variable fragment antibody against c-Met. *Cancer Gene Ther* 2003; 10: 840-849.
19. Liu SX, Sun WS, Cao YL, Ma CH, Han LH, Zhang LN, Wang ZG, Zhu FL. Antisense oligonucleotide targeting at the initiator of hTERT arrests growth of hepatoma cells. *World J Gastroenterol* 2004; 10: 366-370.
20. Jiang Y, Zhou XD, Liu YK, Huang XW, Zhao Y, Xue Q, Sun RX, Chen J, Wu X. Antisense Tcf inhibits the neoplastic growth of liver cancer cells. *J Cancer Res Clin Oncol* 2004; 130: 671-678.
21. Sangkhathat S, Kusafuka T, Miao J, Yoneda A, Nara K, Yamamoto S, Kaneda Y, Fukuzawa M. *In vitro* RNA interference against beta-catenin inhibits the proliferation of pediatric hepatic tumors. *Int J Oncol* 2006; 28: 715-722.
22. Maret A, Galy B, Arnaud E, Bayard F, Prats H. Inhibition of fibroblast growth factor 2 expression by antisense RNA induced a loss of the transformed phenotype in a human hepatoma cell line. *Cancer Res* 1995; 55: 5075-5079.
23. Gu S, Liu CJ, Qiao T, Sun XM, Chen LL, Zhang L. Inhibitory effect of antisense vascular endothelial growth factor 165 eukaryotic expression vector on proliferation of hepatocellular carcinoma cells. *World J Gastroenterol* 2004; 10: 535-539.
24. Wang XH, Li SB, Tong Q, Xie GJ, Wu QM. Effects of adenovirus-mediated human cyclooxygenase-2 antisense RNA on the growth of hepatocellular carcinoma. *World J Gastroenterol* 2005; 11: 6110-6114.
25. Salvi A, Arici B, De Petro G, Barlati S. Small interfering RNA urokinase silencing inhibits invasion and migration of human hepatocellular carcinoma cells. *Mol Cancer Ther* 2004; 3: 671-678.
26. Li H, Fu X, Chen Y, Hong Y, Tan Y, Cao H, Wu M, Wang H. Use of adenovirus-delivered siRNA to target oncoprotein p28GANK in hepatocellular carcinoma. *Gastroenterology* 2005; 128: 2029-2041.

27. Gerolami R, Cardoso J, Lewin M, Bralet MP, Sa Cunha A, Clement O, Brechot C, Tran PL. Evaluation of HSV-tk gene therapy in a rat model of chemically induced hepatocellular carcinoma by intratumoral and intrahepatic artery routes. *Cancer Res* 2000; 60: 993-1001.
28. van Dillen IJ, Mulder NH, Vaalburg W, de Vries EF, Hospers GA. Influence of the bystander effect on HSV-tk/GCV gene therapy. A review. *Curr Gene Ther* 2002; 2: 307-322.
29. Fillat C, Carrio M, Cascante A, Sangro B. Suicide gene therapy mediated by the Herpes Simplex virus thymidine kinase gene/Ganciclovir system: fifteen years of application. *Curr Gene Ther* 2003; 3: 13-26.
30. Qian C, Idoate M, Bilbao R, Sangro B, Bruna O, Vazquez J, Prieto J. Gene transfer and therapy with adenoviral vector in rats with diethylnitrosamine-induced hepatocellular carcinoma. *Hum Gene Ther* 1997; 8: 349-358.
31. Brand K, Arnold W, Bartels T, Lieber A, Kay MA, Strauss M, Dorken B. Liver-associated toxicity of the HSV-tk/GCV approach and adenoviral vectors. *Cancer Gene Ther* 1997; 4: 9-16.
32. Herraiz M, Beraza N, Solano A, Sangro B, Montoya J, Qian C, Prieto J, Bustos M. Liver failure caused by herpes simplex virus thymidine kinase plus ganciclovir therapy is associated with mitochondrial dysfunction and mitochondrial DNA depletion. *Hum Gene Ther* 2003; 14: 463-472.
33. Penuelas I, Mazzolini G, Boan JF, Sangro B, Marti-Climent J, Ruiz M, Ruiz J, Satyamurthy N, Qian C, Barrio JR, Phelps ME, Richter JA, Gambhir SS, Prieto J. Positron emission tomography imaging of adenoviral-mediated transgene expression in liver cancer patients. *Gastroenterology* 2005; 128: 1787-1795.
34. Kievit E, Bershad E, Ng E, Sethna P, Dev I, Lawrence TS, Rehemtulla A. Superiority of yeast over bacterial cytosine deaminase for enzyme/prodrug gene therapy in colon cancer xenografts. *Cancer Res* 1999; 59: 1417-1421.
35. Nyati MK, Symon Z, Kievit E, Dornfeld KJ, Rynkiewicz SD, Ross BD, Rehemtulla A, Lawrence TS. The potential of 5-fluorocytosine/cytosine deaminase enzyme prodrug gene therapy in an intrahepatic colon cancer model. *Gene Ther* 2002; 9: 844-849.
36. Zhang M, Li S, Nyati MK, DeRemer S, Parsels J, Rehemtulla A, Ensminger WD, Lawrence TS. Regional delivery and selective expression of a high-activity yeast cytosine deaminase in an intrahepatic colon cancer model. *Cancer Res* 2003; 63: 658-663.
37. Yazawa K, Fisher WE, Brunicardi FC. Current progress in suicide gene therapy for cancer. *World J Surg* 2002; 26: 783-789.
38. Kan O, Kingsman S, Naylor S. Cytochrome P450-based cancer gene therapy: current status. *Expert Opin Biol Ther* 2002; 2: 857-868.
39. Palmer DH, Mautner V, Mirza D, Oliff S, Gerritsen W, van der Sijp JR, Hubscher S, Reynolds G, Bonney S, Rajaratnam R, Hull D, Horne M, Ellis J, Mountain A, Hill S, Harris PA, Searle PF, Young LS, James ND, Kerr DJ. Virus-directed enzyme prodrug therapy: intratumoral administration of a replication-deficient adenovirus encoding nitroreductase to patients with resectable liver cancer. *J Clin Oncol* 2004; 22: 1546-1552.
40. Kang JH, Chung JK, Lee YJ, Shin JH, Jeong JM, Lee DS, Lee MC. Establishment of a human hepatocellular carcinoma cell line highly expressing sodium iodide symporter for radionuclide gene therapy. *J Nucl Med* 2004; 45: 1571-1576.
41. Faivre J, Clerc J, Gerolami R, Herve J, Longuet M, Liu B, Roux J, Moal F, Perricaudet M, Brechot C. Long-term radioiodine retention and regression of liver cancer after sodium iodide symporter gene transfer in wistar rats. *Cancer Res* 2004; 64: 8045-8051.
42. Abdul-Ghani R, Ohana P, Matouk I, Ayesh S, Ayesh B, Laster M, Bibi O, Giladi H, Molnar-Kimber K, Sughayer MA, de Groot N, Hochberg A. Use of transcriptional regulatory sequences of telomerase (hTER and hTERT) for selective killing of cancer cells. *Mol Ther* 2000; 2: 539-544.
43. Kunitomi M, Takayama E, Suzuki S, Yasuda T, Tsutsui K, Nagaike K, Hiroi S, Tadakuma T. Selective inhibition of hepatoma cells using diphtheria toxin A under the control of the promoter/enhancer region of the human alpha-fetoprotein gene. *Jpn J Cancer Res* 2000; 91: 343-350.
44. Nagane M, Huang HJ, Cavenee WK. The potential of TRAIL for cancer chemotherapy. *Apoptosis* 2001; 6: 191-197.
45. Ma H, Liu Y, Liu S, Xu R, Zheng D. Oral adeno-associated virus-sTRAIL gene therapy suppresses human hepatocellular carcinoma growth in mice. *Hepatology* 2005; 42: 1355-1363.
46. Jacob D, Schumacher G, Bahra M, Davis J, Zhu HB, Zhang LD, Teraishi F, Neuhaus P, Fang BL. Fiber-modified adenoviral vector expressing the tumor necrosis factor-related apoptosis-inducing ligand gene from the human telomerase reverse transcriptase promoter induces apoptosis in human hepatocellular carcinoma cells. *World J Gastroenterol* 2005; 11: 2552-2556.
47. Ye X, Lu Q, Zhao Y, Ren Z, Ren XW, Qiu QH, Tong Y, Liang M, Hu F, Chen HZ. Conditionally replicative adenovirus vector carrying TRAIL gene for enhanced oncolysis of human hepatocellular carcinoma. *Int J Mol Med* 2005; 16: 1179-1184.
48. Sangro B, Melero I, Qian C, Prieto J. Gene therapy of cancer based on interleukin 12. *Curr Gene Ther* 2005; 5: 573-581.
49. Barajas M, Mazzolini G, Genove G, Bilbao R, Narvaiza I, Schmitz V, Sangro B, Melero I, Qian C, Prieto J. Gene therapy of orthotopic hepatocellular carcinoma in rats using adenovirus coding for interleukin 12. *Hepatology* 2001; 33: 52-61.
50. Waehler R, Ittrich H, Mueller L, Krupski G, Ameis D, Schnieders F. Low-dose adenoviral immunotherapy of rat hepatocellular carcinoma using single-chain interleukin-12. *Hum Gene Ther* 2005; 16: 307-317.
51. Sangro B, Mazzolini G, Ruiz J, Herraiz M, Quiroga J, Herrero I, Benito A, Larrache J, Pueyo J, Subtil JC, Olague C, Sola J, Sadaba B, Lacasa C, Melero I, Qian C, Prieto J. Phase I trial of intratumoral injection of an adenovirus encoding interleukin-12 for advanced digestive tumors. *J Clin Oncol* 2004; 22: 1389-1397.
52. Wang L, Hernandez-Alcoceba R, Shankar V, Zabala M, Kochanek S, Sangro B, Kramer MG, Prieto J, Qian C. Prolonged and inducible transgene expression in the liver using gutless adenovirus: a potential therapy for liver cancer. *Gastroenterology* 2004; 126: 278-289.
53. Lieschke GJ, Rao PK, Gately MK, Mulligan RC. Bioactive murine and human interleukin-12 fusion proteins which retain antitumor activity *in vivo*. *Nat Biotechnol* 1997; 15: 35-40.
54. Chen WY, Cheng YT, Lei HY, Chang CP, Wang CW, Chang MS. IL-24 inhibits the growth of hepatoma cells in vivo. *Genes Immun* 2005; 6: 493-499. [PMID: 15959534]
55. Gupta P, Su ZZ, Lebedeva IV, Sarkar D, Sauane M, Emdad L, Bachelor MA, Grant S, Curiel DT, Dent P, Fisher PB. mda-7/IL-24: Multifunctional cancer-specific apoptosis-inducing cytokine. *Pharmacol Ther* 2006; 111: 596-628
56. Martinet O, Ermekova V, Qiao JQ, Sauter B, Mandeli J, Chen L, Chen SH. Immunomodulatory gene therapy with interleukin 12 and 4-1BB ligand: long- term remission of liver metastases in a mouse model. *J Natl Cancer Inst* 2000; 92: 931-936.
57. Putzer BM, Stiewe T, Rodicker F, Schildgen O, Ruhm S, Dirsch O, Fiedler M, Damen U, Tennant B, Scherer C, Graham FL, Roggendorf M. Large nontransplanted hepatocellular carcinoma in woodchucks: treatment with adenovirus-mediated delivery of interleukin 12/B7.1 genes. *J Natl Cancer Inst* 2001; 93: 472-479.
58. Schmitz V, Barajas M, Wang L, Peng D, Duarte M, Prieto J, Qian C. Adenovirus-mediated CD40 ligand gene therapy in a rat model of orthotopic hepatocellular carcinoma. *Hepatology* 2001; 34: 72-81.
59. Narvaiza I, Mazzolini G, Barajas M, Duarte M, Zaratiegui M, Qian C, Melero I, Prieto J. Intratumoral coinjection of two adenoviruses, one encoding the chemokine IFN-gamma-inducible protein-10 and another encoding IL-12, results in marked antitumoral synergy. *J Immunol* 2000; 164: 3112-3122.
60. Comes A, Di Carlo E, Musiani P, Rosso O, Meazza R, Chiodoni C, Colombo MP, Ferrini S. IFN-gamma-independent synergistic effects of IL-12 and IL-15 induce anti-tumor immune responses in syngeneic mice. *Eur J Immunol* 2002; 32: 1914-1923.

61. Wang Z, Qiu SJ, Ye SL, Tang ZY, Xiao X. Combined IL-12 and GM-CSF gene therapy for murine hepatocellular carcinoma. *Cancer Gene* Ther 2001; 8: 751-758.
62. Mazzolini G, Narvaiza I, Martinez-Cruz LA, Arina A, Barajas M, Galofre JC, Qian C, Mato JM, Prieto J, Melero I. Pancreatic cancer escape variants that evade immunogene therapy through loss of sensitivity to IFNgamma-induced apoptosis. *Gene Ther* 2003; 10: 1067-1078.
63. Alves A, Vibert E, Trajcevski S, Solly S, Fabre M, Soubrane O, Qian C, Prieto J, Klatzmann D, Panis Y. Adjuvant interleukin-12 gene therapy for the management of colorectal liver metastases. *Cancer Gene Ther* 2004; 11: 782-789.
64. Stefani AL, Barzon L, Castagliuolo I, Guido M, Pacenti M, Parolin C, Farinati F, Palu G. Systemic efficacy of combined suicide/cytokine gene therapy in a murine model of hepatocellular carcinoma. *J Hepatol* 2005; 42: 728-735.
65. Grimm CF, Ortmann D, Mohr L, Michalak S, Krohne TU, Meckel S, Eisele S, Encke J, Blum HE, Geissler M. Mouse alpha-fetoprotein-specific DNA-based immunotherapy of hepatocellular carcinoma leads to tumor regression in mice. *Gastroenterology* 2000; 119: 1104-1112.
66. Saeki A, Nakao K, Nagayama Y, Yanagi K, Matsumoto K, Hayashi T, Ishikawa H, Hamasaki K, Ishii N, Eguchi K. Diverse efficacy of vaccination therapy using the alpha-fetoprotein gene against mouse hepatocellular carcinoma. *Int J Mol Med* 2004; 13: 111-116.
67. Peron JM, Couderc B, Rochaix P, Douin-Echinard V, Asnacios A, Souque A, Voigt JJ, Buscail L, Vinel JP, Favre G. Treatment of murine hepatocellular carcinoma using genetically modified cells to express interleukin-12. *J Gastroenterol Hepatol* 2004; 19: 388-396.
68. Ge NL, Ye SL, Zheng N, Sun RX, Liu YK, Tang ZY. Prevention of hepatocellular carcinoma in mice by IL-2 and B7-1 genes co-transfected liver cancer cell vaccines. *World J Gastroenterol* 2003; 9: 2182-2185.
69. Vollmer CM, Jr., Eilber FC, Butterfield LH, Ribas A, Dissette VB, Koh A, Montejo LD, Lee MC, Andrews KJ, McBride WH, Glaspy JA, Economou JS. Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma. *Cancer Res* 1999; 59: 3064-3067.
70. Melero I, Duarte M, Ruiz J, Sangro B, Galofre J, Mazzolini G, Bustos M, Qian C, Prieto J. Intratumoral injection of bone-marrow derived dendritic cells engineered to produce interleukin-12 induces complete regression of established murine transplantable colon adenocarcinomas. *Gene Ther* 1999; 6: 1779-1784.
71. Zhu M, Terasawa H, Gulley J, Panicali D, Arlen P, Schlom J, Tsang KY. Enhanced activation of human T cells via avipox vector-mediated hyperexpression of a triad of costimulatory molecules in human dendritic cells. *Cancer Res* 2001; 61: 3725-3734.
72. Tirapu I, Rodriguez-Calvillo M, Qian C, Duarte M, Smerdou C, Palencia B, Mazzolini G, Prieto J, Melero I. Cytokine gene transfer into dendritic cells for cancer treatment. *Curr Gene Ther* 2002; 2: 79-89.
73. Mazzolini G, Alfaro C, Sangro B, Feijoo E, Ruiz J, Benito A, Tirapu I, Arina A, Sola J, Herraiz M, Lucena F, Olague C, Subtil J, Quiroga J, Herrero I, Sadaba B, Bendandi M, Qian C, Prieto J, Melero I. Intratumoral injection of dendritic cells engineered to secrete interleukin-12 by recombinant adenovirus in patients with metastatic gastrointestinal carcinomas. *J Clin Oncol* 2005; 23: 999-1010.
74. Feijoo E, Alfaro C, Mazzolini G, Serra P, Penuelas I, Arina A, Huarte E, Tirapu I, Palencia B, Murillo O, Ruiz J, Sangro B, Richter JA, Prieto J, Melero I. Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8. *Int J Cancer* 2005; 116: 275-281.
75. Mazzolini G, Qian C, Narvaiza I, Barajas M, Borras-Cuesta F, Xie X, Duarte M, Melero I, Prieto J. Adenoviral gene transfer of interleukin 12 into tumors synergizes with adoptive T cell therapy both at the induction and effector level. *Hum Gene Ther* 2000; 11: 113-125.
76. Hong SY, Lee MH, Kim KS, Jung HC, Roh JK, Hyung WJ, Noh SH, Choi SH. Adeno-associated virus mediated endostatin gene therapy in combination with topoisomerase inhibitor effectively controls liver tumor in mouse model. *World J Gastroenterol* 2004; 10: 1191-1197.
77. Goldman CK, Kendall RL, Cabrera G, Soroceanu L, Heike Y, Gillespie GY, Siegal GP, Mao X, Bett AJ, Huckle WR, Thomas KA, Curiel DT. Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis, and mortality rate. *Proc Natl Acad Sci U S A* 1998; 95: 8795-8800.
78. Lin P, Buxton JA, Acheson A, Radziejewski C, Maisonpierre PC, Yancopoulos GD, Channon KM, Hale LP, Dewhirst MW, George SE, Peters KG. Antiangiogenic gene therapy targeting the endothelium-specific receptor tyrosine kinase Tie2. *Proc Natl Acad Sci U S A* 1998; 95: 8829-8834.
79. Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 1999; 285: 245-248.
80. Matsumoto K, Ishikawa H, Nishimura D, Hamasaki K, Nakao K, Eguchi K. Antiangiogenic property of pigment epithelium-derived factor in hepatocellular carcinoma. *Hepatology* 2004; 40: 252-259.
81. Murakami M, Nagai E, Mizumoto K, Saimura M, Ohuchida K, Inadome N, Matsumoto K, Nakamura T, Maemondo M, Nukiwa T, Tanaka M. Suppression of metastasis of human pancreatic cancer to the liver by transportal injection of recombinant adenoviral NK4 in nude mice. *Int J Cancer* 2005; 117: 160-165.
82. Harada N, Shimada M, Okano S, Suehiro T, Soejima Y, Tomita Y, Maehara Y. IL-12 gene therapy is an effective therapeutic strategy for hepatocellular carcinoma in immunosuppressed mice. *J Immunol* 2004; 173: 6635-6644.
83. Mullen JT, Tanabe KK. Viral oncolysis for malignant liver tumors. *Ann Surg Oncol* 2003; 10: 596-605.
84. Parato KA, Senger D, Forsyth PA, Bell JC. Recent progress in the battle between oncolytic viruses and tumours. *Nat Rev Cancer* 2005; 5: 965-976.
85. Fueyo J, Gomez-Manzano C, Alemany R, Lee PS, McDonnell TJ, Mitlianga P, Shi YX, Levin VA, Yung WK, Kyritsis AP. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect *in vivo*. *Oncogene* 2000; 19: 2-12
86. Heise C, Hermiston T, Johnson L, Brooks G, Sampson-Johannes A, Williams A, Hawkins L, Kirn D. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nat Med* 2000; 6: 1134-1139.
87. Ko D, Hawkins L, Yu DC. Development of transcriptionally regulated oncolytic adenoviruses. *Oncogene* 2005; 24: 7763-7774.
88. Varghese S, Rabkin SD. Oncolytic herpes simplex virus vectors for cancer virotherapy. *Cancer Gene Ther* 2002; 9: 967-978.
89. Mullen JT, Kasuya H, Yoon SS, Carroll NM, Pawlik TM, Chandrasekhar S, Nakamura H, Donahue JM, Tanabe KK. Regulation of herpes simplex virus 1 replication using tumor-associated promoters. *Ann Surg* 2002; 236: 502-512; discussion 512-503.
90. Hermiston TW, Kuhn I. Armed therapeutic viruses: strategies and challenges to arming oncolytic viruses with therapeutic genes. *Cancer Gene Ther* 2002; 9: 1022-1035.
91. O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A, Boyle L, Pandey K, Soria C, Kunich J, Shen Y, Habets G, Ginzinger D, McCormick F. Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. *Cancer* Cell 2004; 6: 611-623.
92. Vollmer CM, Ribas A, Butterfield LH, Dissette VB, Andrews KJ, Eilber FC, Montejo LD, Chen AY, Hu B, Glaspy JA, McBride WH, Economou JS. p53 selective and nonselective replication of an E1B-deleted adenovirus in hepatocellular carcinoma. *Cancer Res* 1999; 59: 4369-4374.

93. Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C, Randlev B, Gillenwater AM, Bruso P, Kaye SB, Hong WK, Kirn DH. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med* 2000; 6: 879-885.
94. Habib N, Salama H, Abd El Latif Abu Median A, Isac Anis I, Abd Al Aziz RA, Sarraf C, Mitry R, Havlik R, Seth P, Hartwigsen J, Bhushan R, Nicholls J, Jensen S. Clinical trial of E1B-deleted adenovirus (dl1520) gene therapy for hepatocellular carcinoma. *Cancer Gene Ther* 2002; 9: 254-259.
95. Hamid O, Varterasian ML, Wadler S, Hecht JR, Benson A, 3rd, Galanis E, Uprichard M, Omer C, Bycott P, Hackman RC, Shields AF. Phase II trial of intravenous CI-1042 in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; 21: 1498-1504.
96. Makower D, Rozenblit A, Kaufman H, Edelman M, Lane ME, Zwiebel J, Haynes H, Wadler S. Phase II clinical trial of intralesional administration of the oncolytic adenovirus ONYX-015 in patients with hepatobiliary tumors with correlative p53 studies. *Clin Cancer Res* 2003; 9: 693-702.
97. Reid T, Galanis E, Abbruzzese J, Sze D, Wein LM, Andrews J, Randlev B, Heise C, Uprichard M, Hatfield M, Rome L, Rubin J, Kirn D. Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints. *Cancer Res* 2002; 62: 6070-6079.
98. Takahashi M, Sato T, Sagawa T, Lu Y, Sato Y, Iyama S, Yamada Y, Fukaura J, Takahashi S, Miyanishi K, Yamashita T, Sasaki K, Kogawa K, Hamada H, Kato J, Niitsu Y. E1B-55K-deleted adenovirus expressing E1A-13S by AFP-enhancer/promoter is capable of highly specific replication in AFP-producing hepatocellular carcinoma and eradication of established tumor. *Mol Ther* 2002; 5: 627-634.
99. Li Y, Yu DC, Chen Y, Amin P, Zhang H, Nguyen N, Henderson DR. A hepatocellular carcinoma-specific adenovirus variant, CV890, eliminates distant human liver tumors in combination with doxorubicin. *Cancer Res* 2001; 61: 6428-6436.
100. Sagawa T, Takahashi M, Sato T, Sato Y, Lu Y, Sumiyoshi T, Yamada Y, Iyama S, Fukaura J, Sasaki K, Hamada H, Miyanishi K, Takayama T, Kato J, Niitsu Y. Prolonged survival of mice with multiple liver metastases of human colon cancer by intravenous administration of replicable E1B-55K-deleted adenovirus with E1A expressed by CEA promoter. *Mol Ther* 2004; 10: 1043-1050.
101. Wirth T, Zender L, Schulte B, Mundt B, Plentz R, Rudolph KL, Manns M, Kubicka S, Kuhnel F. A telomerase-dependent conditionally replicating adenovirus for selective treatment of cancer. *Cancer Res* 2003; 63: 3181-3188.
102. Kim E, Kim JH, Shin HY, Lee H, Yang JM, Kim J, Sohn JH, Kim H, Yun CO. Ad-mTERT-delta19, a conditional replication-competent adenovirus driven by the human telomerase promoter, selectively replicates in and elicits cytopathic effect in a cancer cell-specific manner. *Hum Gene Ther* 2003; 14: 1415-1428.
103. Jakubczak JL, Ryan P, Gorziglia M, Clarke L, Hawkins LK, Hay C, Huang Y, Kaloss M, Marinov A, Phipps S, Pinkstaff A, Shirley P, Skripchenko Y, Stewart D, Forry-Schaudies S, Hallenbeck PL. An oncolytic adenovirus selective for retinoblastoma tumor suppressor protein pathway-defective tumors: dependence on E1A, the E2F-1 promoter, and viral replication for selectivity and efficacy. *Cancer Res* 2003; 63: 1490-1499.
104. Bristol JA, Zhu M, Ji H, Mina M, Xie Y, Clarke L, Forry-Schaudies S, Ennist DL. *In vitro* and *in vivo* activities of an oncolytic adenoviral vector designed to express GM-CSF. *Mol Ther* 2003; 7: 755-764.
105. Sova P, Ren XW, Ni S, Bernt KM, Mi J, Kiviat N, Lieber A. A tumor-targeted and conditionally replicating oncolytic adenovirus vector expressing TRAIL for treatment of liver metastases. *Mol Ther* 2004; 9: 496-509.
106. Pei Z, Chu L, Zou W, Zhang Z, Qiu S, Qi R, Gu J, Qian C, Liu X. An oncolytic adenoviral vector of Smac increases antitumor activity of TRAIL against HCC in human cells and in mice. *Hepatology* 2004; 39: 1371-1381.
107. Zhang ZL, Zou WG, Luo CX, Li BH, Wang JH, Sun LY, Qian QJ, Liu XY. An armed oncolytic adenovirus system, ZD55-gene, demonstrating potent antitumoral efficacy. *Cell Res* 2003; 13: 481-489.
108. Li GC, Yang JM, Nie MM, Su CG, Sun LC, Qian YZ, Fang GE, Sham J, Wu MC, Qian QJ. Potent antitumoral effects of a novel gene-viral therapeutic system CNHK300-mEndostatin in hepatocellular carcinoma. *Chin Med J* (Engl) 2005; 118: 179-185.
109. Epstein AL, Marconi P, Argnani R, Manservigi R. HSV-1-derived recombinant and amplicon vectors for gene transfer and gene therapy. *Curr Gene Ther* 2005; 5: 445-458.
110. Song TJ, Eisenberg DP, Adusumilli PS, Hezel M, Fong Y. Oncolytic herpes viral therapy is effective in the treatment of hepatocellular carcinoma cell lines. *J Gastrointest Surg* 2006; 10: 532-542.
111. Pawlik TM, Nakamura H, Mullen JT, Kasuya H, Yoon SS, Chandrasekhar S, Chiocca EA,Tanabe KK. Prodrug bioactivation and oncolysis of diffuse liver metastases by a herpes simplex virus 1 mutant that expresses the CYP2B1 transgene. *Cancer* 2002; 95: 1171-1181
112. Nakamura H, Mullen JT, Chandrasekhar S, Pawlik TM, Yoon SS, Tanabe KK. Multimodality therapy with a replication-conditional herpes simplex virus 1 mutant that expresses yeast cytosine deaminase for intratumoral conversion of 5-fluorocytosine to 5-fluorouracil. *Cancer Res* 2001; 61: 5447-5452.
113. Jarnagin WR, Zager JS, Klimstra D, Delman KA, Malhotra S, Ebright M, Little S, DeRubertis B, Stanziale SF, Hezel M, Federoff H, Fong Y. Neoadjuvant treatment of hepatic malignancy: an oncolytic herpes simplex virus expressing IL-12 effectively treats the parent tumor and protects against recurrence-after resection. *Cancer Gene Ther* 2003; 10: 215-223.
114. Shinozaki K, Ebert O, Kournioti C, Tai YS, Woo SL. Oncolysis of multifocal hepatocellular carcinoma in the rat liver by hepatic artery infusion of vesicular stomatitis virus. *Mol Ther* 2004; 9: 368-376.