Recent Advances in Esophageal Cancer Gene Therapy

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In recent years, the development of gene therapy systems as new treatment or prevention strategies for various malignant diseases has been explored. Based on the genetic background of esophageal cancer, several molecular therapies have been developed. In this article, we review p53 genetic alterations and angiogenesis in esophageal cancer. Our recent clinical results from a phase I/II study of adenoviral-mediated p53 gene therapy for patients with unresectable esophageal cancer are reviewed. Lastly we review the available experimental therapeutic models from the literature and our experimental work over the past few years are reviewed. (Ann Thorac Cardiovasc Surg 2008; 14: 3–8)

Key words: p53, angiogenesis, gene therapy, esophageal cancer, clinical study

Introduction

Because surgical resection alone rarely results in long-term survival for advanced esophageal cancer,1) much effort is now focused on combined multi-modality treatments in an attempt to improve local control of tumor growth and to eliminate micro-metastasis present at the time of surgery. Recently, the use of neoadjuvant chemoradiotherapy followed by esophagectomy has become a widespread approach due to several favorable reports.2,3) However, the prognosis remains extremely poor in unresectable patients.4) In this context, it will be necessary to enhance the anti-tumor efficacy of chemotherapy and radiotherapy in order to improve the outcome of the multidisciplinary therapy.

Molecular Targets in Esophageal Cancer Treatment

p53 abnormalities are observed in 40–60% of patients with esophageal cancer, even in an early precancerous lesion.5,6) Indeed, p53 genetic alteration is a good predictor for treatment responses and survival in esophageal cancer.7–10) Genetic analysis can be used as a prognostic marker for esophageal carcinoma. p53 genotyping may define a subset of patients who respond to chemotherapy/radiotherapy and may predict who potentially benefit from multimodality therapy.7)

Angiogenic and growth factors also play an essential role in the process of growth, metastasis and treatment resistance of esophageal carcinoma.11) Among several angiogenic factors, vascular endothelial growth factor (VEGF) has been shown to be vital for resistance to treatment.12) Immunohistochemical analyses of esophageal carcinomas have revealed that VEGF expression, dThdPase expression, and angiogenesis are good predictors for treatment responses and survival in esophageal cancer.13–16) Based on reports that indicate a significant association between angiogenesis and p53 expression, it is anticipated that wild type p53 gene transfer into cancer cells may down-regulate angiogenesis. Hepatocyte growth factor (HGF) also contributes to invasion and metastasis of esophageal carcinoma cells (unpublished data).

Clinical Trials of p53 Gene Therapy

In preclinical tumor models, p53 gene transfer as mediated by retroviral or adenoviral expression vectors restored drug and radiation sensitivity or directly induced...
Individual doses were 10 to 25 among those who were not subjected to radical surgery. Among patients who could not undergo radical surgery, 10 to 11 viral particles (VP) were administered on days 1 and 3, and the patients were treated for up to five cycles.

A total of 10 patients were enrolled in this trial (Table 1). On commencement of gene therapy, five patients had tumor invasion into adjacent organs (Patient 3, 4, 7, 9, and 10) and four had high risk factors for surgery (Patients 1, 2, 5, and 8). The remaining patient had a T3 tumor with multiple lymph node metastases (Patient 6). Eight patients had tumors with p53 mutations among exons 5 to 8. Treatment cycles ranged from one to five cycles, with two being the median number of cycles. Patient 6 did not undergo a second cycle of the treatment because he showed rapid progression of distant metastases after the first cycle. The most common adverse events were fever and local pain (NCI grade 1 or 2). Fever was observed in all patients and pain in 30%. Overall, drug administration was feasible and well tolerated.

Nine of the 10 eligible patients have died of the disease. One patient had local tumor progression and four patients had systemic progression during the initial 56 days. Six of 10 patients were alive for more than one year (Table 1). Three patients showed no evidence of tumor on multiple biopsies after the treatment (Patients 2, 3, and 5). Patient 2 had a tumor with a deep ulcer before the treatment. After three or four cycles, the ulcer had changed to a mild shape. No viable cancer cells were observed in biopsy specimens. Stable local tumor and no residual squamous cell carcinoma (SCC) were found on esophageal biopsies during the five cycles of the treatment. Patient 3 had a large 50 mm mass and was unable to swallow at the initiation of gene therapy. After two injections of Ad5CMV-p53, he was able to swallow liquid and meals. Although the endoscope could not pass through the stenotic portion of the lesion before the treatment, it passed through after the treatment. Patient 5 did not show tumor progression for 24 months after initial gene therapy. Although a few viable cancer cells were observed in biopsy specimens at 24 months, the tumor was well controlled by argon-plasma coagulation. He currently remains progression-free and alive 65 months after completion of the treatment. The overall survival rate was 60% at one year. Four patients have developed clinically diagnosed distant metastases (two cases of bone metastasis, one of liver and lymph node metastasis, and one of lung metastasis). Three of these four patients with development of distant metastases died of the disease within 3 months after initial viral treatment. The median time to local as well as distant progression was 6 months (Fig. 1).

Our clinical results demonstrate that administration of Ad5CMV-p53 via intratumoral injection is feasible, safe and well-tolerated in a population of patients with chemoradiation resistant esophageal SCC. Acute NCI grade 1 or 2 toxicities, including local pain and fever, were acceptable and provided further evidence of biological activity from the treatment. Transduction of the vector-p53 gene into tumor tissue was confirmed by vector-specific DNA-PCR analysis in all patients’ biopsy specimens. Although 80% of patients showed increased p53 mRNA after treatment and 75% of these showed elevated p21 and MDM2 downstream markers, the increase of RT-PCR signals was more minor than we expected. This could partly be explained by the differences in bi-
All patients were evaluated for evidence of antitumor activity and clinical benefit. Six patients the disease was established both the local site as well as systemically and survived more than one year after starting treatment. Among these six patients, one is alive as of today, more than 5 years after completion of gene therapy.

### Future Clinical Trial of p53 Gene Therapy Combined with Chemo-Radiotherapy or Immunotherapy

According to recent reports, adenovirus-mediated p53 gene transfer is frequently used, together with cis-dichloro-diammineplatinum(II) administration or ionizing radiation. A phase II trial of adenovirus-mediated p53 gene transfer in conjunction with radiation therapy or chemotherapy for lung cancer patients was safe and effective. Because treatment response is strongly associated with survival, a combination treatment with standard chemoradiation and Ad5CMV-p53 may be an attractive modality. Further preclinical studies are required to develop a new combination therapy with CDDP, 5-FU, and Ad5CMV-p53, and also a combination with concurrent chemoradiotherapy (Fig. 2).

Another promising combination treatment with p53 gene transfer is dendritic cell (DC) therapy. Recently, the immunologic and clinical effects of a cancer vaccine consisting of DC transduced with the full-length wild-type p53 gene delivered via an adenoviral vector in patients with extensive stage small cell lung cancer were reported. They reported that p53-specific T cell responses to vaccination were observed in 57.1% of patients. They observed a high rate of objective clinical responses to chemotherapy (61.9%) that immediately followed vaccination. They supposed that optimal use of vaccination might be more effective, not as a separate modality, but in direct combination with chemotherapy.

### Anti-Angiogenesis Gene Therapy

Based on the fact that VEGF, HGF, and other angiogenic factors play a definite role in tumor invasion and metastasis in esophageal carcinoma, anti-angiogenic gene
therapy might also be a favorable approach against advanced stage esophageal carcinoma. Recently, NK4, four-kringles containing an intra-molecular fragment of HGF was isolated as a competitive antagonist for the HGF-c-Met system. Independent of its HGF-antagonist action, NK4 inhibited angiogenesis induced by VEGF and basic fibroblast growth factor, as well as HGF, indicating that NK4 is a bifunctional molecule that acts as an HGF-antagonist and angiogenesis inhibitor. In experimental models of distinct types of cancers, NK4 protein administration or NK4 gene therapy inhibited tumor invasion, metastasis, and angiogenesis-dependent tumor growth. Esophageal carcinoma cell treatment with NK4 also proved to induce malignant tumors to be “static” in both tumor growth and spreading. Based on the preclinical studies of NK4 gene delivery, a Phase I/II study was approved by the Chiba University Graduate School of Medicine Institutional Review Board.

Double Gene Transfer to Enhance Single Gene Therapy

Some reports have shown that double gene transfer enhanced the anti-tumor activity of single gene transfer. They suggested that p14, p16, and p33 genes were able to enhance apoptotic tumor cell death with p53 gene therapy. We also suggested a role for mdm2 in the synergistic effects observed in combined p33 and p53 gene transfer into esophageal carcinoma cells. Although stable expression of both genes in the same tumor cells was supposedly achieved for double gene therapy, this mode of gene therapy should undergo clinical study in the near future.

Oncolytic Virus Therapy

Conventional cancer gene therapy, even if considered to temporarily suppress tumor growth, does not inhibit extended tumor growth and distant metastases. A phase II trial, using an E1B-deleted adenovirus (ONYX-015) designed to selectively lyse p53-deficient cancer cells, was reported in which the virus was administered to patients via a direct intratumoral injection for untreated oral squamous cell carcinoma. They demonstrated that the virus replicates selectively in the tumor as opposed to normal tissue after this direct injection. No adverse effects of the viral injection were noted. Those data supported the concept that ONYX-015 is replication deficient in normal, compared with cancerous, tissues and has potential as a selective anticancer agent against tumor tissues.

Therefore, we examined the potential clinical feasibility of virus-mediated tumor destruction, which can further suppress tumor growth. We showed that human tumors were more susceptible to Ad in which the E1A expression was controlled by a putative tumor promoter rather than normal cells, and that a replication of the Ad was greater in tumor cells than in normal cells. The promoter region of the midkine gene was a good candidate to drive therapeutic genes in a tumor specific manner through conditionally replicative adenovirus.

Immunology Based Gene Therapy

Antitumor immune responses are initiated after the acquisition of putative tumor antigens by dendritic cells (DCs). Therefore, enhanced antigen presentation is a crucial step for the early phase of cell-mediated immunity. Destruction of tumors can release the tumor antigens and DCs come to recognize them thereafter. Activation of DCs induced production of a number of cytokines, and we showed that the interleukin-2 or 12 family secreted from tumors could induce systemic antitumor immunity. DC-based immunotherapy has been clinically evaluated, but it still requires modification to improve the outcomes. We previously demonstrated that F-gene–deleted non-transmissible Sendai virus (SeV/dF)–activated DCs (DCs/SeV/dF) have strong antitumor effects against squamous cell carcinoma. SeV/dF shows high transfection efficiency to DCs and leads them to up-regulate co-stimulatory molecules. Intratumoral injection of DCs/SeV/dF resulted in a marked and representative inhibition of the tumor, even when the tumors were well-vascularized. Results from these preclinical studies are promising for further clinical trials to evaluate the best combination of molecular approaches against esophageal carcinoma.
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References


