Review Article

Interferon-β gene therapy for cancer: Basic research to clinical application

Jun Yoshida,[1] Masaaki Mizuno[2] and Toshihiko Wakabayashi[1]

Departments of [1]Neurosurgery and [2]Molecular Neurosurgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550.

(Received July 4 2003/Revised July 1, 2004/2nd Revised September 25, 2004/Accepted September 27, 2004)

Interferon-β gene therapy for cancer is the first such protocol developed in Japan. Here we describe the development process of our interferon-β gene therapy from basic research to clinical application. Interestingly, the biological and biochemical characteristics of interferon-β gene therapy through transfer of the interferon-β gene into tumor cells by means of cationic liposomes differed from those of conventional interferon-β protein therapy. Interferon-β gene transfer could induce apoptosis in interferon-β protein-resistant tumor cells, such as glioma, melanoma, and renal cell carcinoma. Induction of apoptosis was related to the level of intracellular mRNA of interferon-β, prolongation of the phosphorylation time of molecules in the interferon-β signal transduction pathway, such as JAK1, Trk2, and STAT1, and activation of DNase γ. In our preclinical study we developed lyophilized cationic liposomes containing interferon-β gene (gene drug) for clinical use and confirmed their safety. Thereafter, we performed a pilot clinical trial in patients with malignant glioma and confirmed the safety and effectiveness of this interferon-β gene therapy. In this review we also comment on the status of gene therapy regulation in Japan. Interferon-β gene therapy is expected to become widely available for clinical use in cancer patients, and this new strategy might be extended to molecular targeting therapy, or used in combination with cell therapy or other therapies. (Cancer Sci 2004; 95: 858–865)

Since Watson and Click discovered the double-stranded structure of DNA in 1953, recombinant DNA technology and molecular biology have developed rapidly, and the complete sequence of the human genome, consisting of 3 billion base pairs, has been identified through the human genome project (1990–2003). This has opened the way for a new generation of advanced technology-based medicine, including gene medicine (genetic diagnosis and gene therapy), regenerative medicine, robotic medicine, molecular medicine, and nanomedicine. In particular, gene therapy offers tremendous promise for the future treatment of cancer.

The first gene therapy was initiated on September 14, 1990 in the USA for a patient with severe combined immunodeficiency (adenosine deaminase [ADA] deficiency). Since then, there have been more than 600 trials worldwide, and more than 4000 patients have received some kind of gene therapy. Recently, the target diseases have been extended from congenital metabolic disorders to malignant tumors which cannot be cured by existing treatments, and even chronic benign diseases which result in a poor quality of life.

In Japan, gene therapy for ADA deficiency began in 1995 at Hokkaido University Hospital using the same protocol as in the USA. To date, twenty gene therapy protocols have been developed. Among them, fifteen are related to cancers. Targeted diseases include renal cell carcinoma, lung cancer (non-small cell carcinoma), esophageal cancer, breast cancer, prostate cancer, brain tumor (malignant glioma), leukemia, and colon cancer. In the Department of Neurosurgery and Molecular Neurosurgery, Nagoya University Graduate School of Medicine, we have started a gene therapy protocol of our own since April 2000, an interferon (IFN)-β gene therapy using cationic liposomes. This protocol is the first using made-in-Japan technology. Here we summarize the developmental process of our human IFN-β gene therapy for patients with malignant glioma.

1. Basic studies

Malignant gliomas are too invasive to cure with surgical resection alone. In general, patients with malignant glioma undergo adjuvant therapy, including radiation therapy, chemotherapy, and immunological therapy after surgical resection, but their prognosis remains poor. Gene therapy is a much-awaited new strategy to overcome the misery associated with this neoplasm. However, only a few gene therapies have been directed toward diseases of the central nervous system (CNS), because the CNS is a very complex and critical organ, and access to it is restricted by the blood-brain barrier (BBB). It is therefore important to develop an appropriate delivery system. We currently use a liposomal gene delivery system.

1-1 Liposomes as a gene delivery system

Liposomes are artificial lipid bilayer vesicles considered to be useful as a drug delivery system. They can be morphologically divided into three types; small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), and multilamellar vesicles (MLV) (Fig. 1A). In the 1980s, it was well established that liposomes bearing positive charges on their surface could provide more efficient delivery of their entrapped components into cells, compared to other types of liposomes.[1,2] Our research on cationic liposomes was initiated in 1988. We found, in collaboration with Yagi and his colleagues, that cationic liposomes consisting of *N*-(α-trimethylammonioacetyl)didodecyl-D-glutamate chloride (TMAG), dilauroyl phosphatidylcholine (DLPC), and dioleoyl phosphatidylethanolamine (DOPE) (1:2:2 or 1:2:3, molar ratio) provide high-efficiency DNA entrapment and a high potential for DNA transfer to human glioma cells.[3–8] Moreover, DNA/liposomes are one of the safest delivery modalities to the CNS. Shin *et al*. reported that delivery of genes via DNA/liposome complexes to the brain could be achieved by incorporating antibodies to the transferrin receptor in order to facilitate passage across the BBB.[9]

Recently, various modifications of liposomes, e.g., combination of adeno-associated virus (AAV) vectors, adenovirus vectors, and nanotechnology-based molecules (Fig. 1B),[10–12] have been investigated.

jyoshida@med.nagoya-u.ac.jp

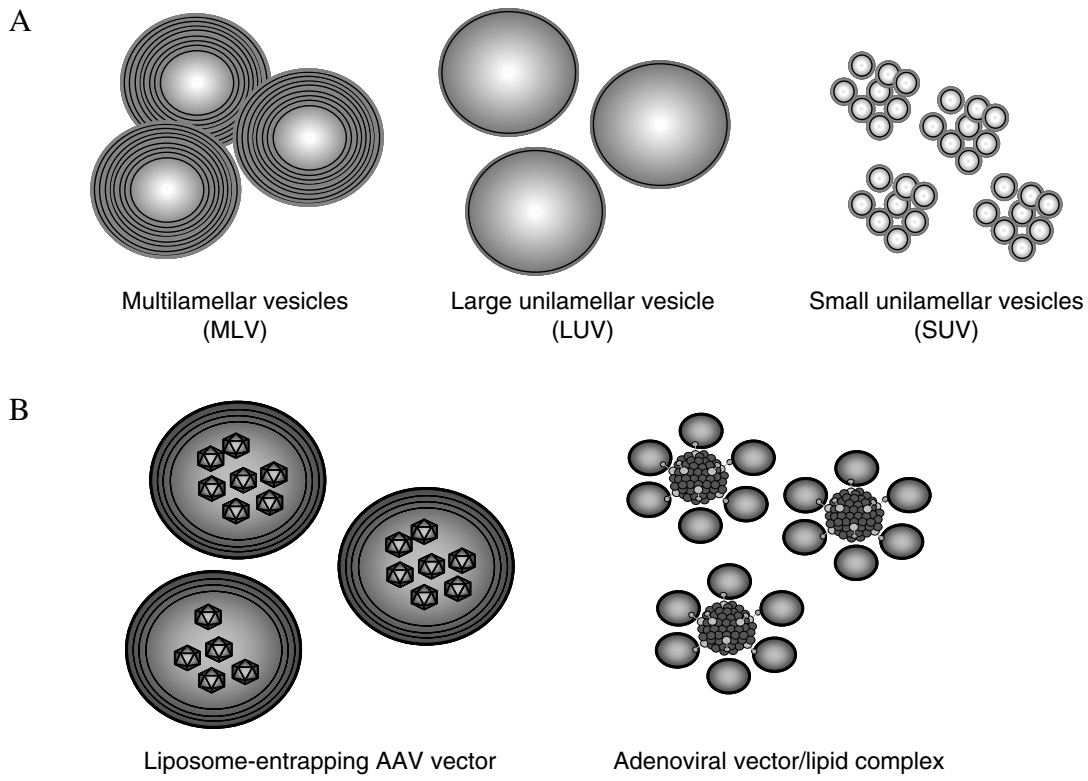


Fig. 1. Cationic liposomes and their application. A. Cationic liposomes can be morphologically divided into three types: small unilamellar vesicles (SUVs); large unilamellar vesicles (LUVs); and multilamellar vesicles (MLVs). SUVs bind the genes to their surfaces, producing DNA-lipid complexes. SUVs have already been applied to clinical cancer gene therapy, especially immuno-gene therapy. LUVs and MLVs generally entrap the genes within the liposomes, rather than on the surfaces. MLVs can retain transfection efficacy for more than 1 year. B. Cationic liposomes have high potential for a variety of modifications. For example, they have a higher potential to combine with other materials such as viral vectors, antibodies, and other therapeutic agents. In our university, we are striving to develop combined therapy with liposomes and adeno-associated virus (AAV) vectors or adenovirus vectors. AAV vector-associated liposomes have more than 10-fold greater transduction efficiency than liposomes containing plasmid DNA and more than 6-fold greater than AAV vector alone. In contrast, adenoviral vector/lipid complex reduces the antigenicity of the adenoviral vector *in vivo* without diminishing the antitumor activity.

1-2 IFN-β

Human IFN-β is thought to be an important factor in the growth of human glioma and melanoma because homozygous deletions of the class I IFN gene cluster, comprising multiple IFN-α genes and a single IFN-β gene, have been demonstrated in these tumors.[13, 14] In the 1980s, IFN-β protein was clinically used as an anticancer drug in Japan, and it showed a clear growth-inhibitory effect on malignant glioma and melanoma.[15, 16] However, tumor regression was observed in only 10–30% and 15–20% of the patients treated for glioma and melanoma, respectively, and survival prolongation was not attained in either.[17] IFN-β protein also deserves attention as a cell-cycle regulator, inducing aberrant cell-cycle progression, which occurs predominantly as S phase accumulation, and less frequently as other cell-cycle effects, such as G1 arrest, or the entry of tumor cells into a senescent-like state.[18]

1-3 Antitumor mechanisms of IFN-β gene transfer

We investigated the antitumor activity of IFN-β gene therapy in both *in vitro* and *in vivo* experiments. In the *in vivo* experiments, human glioma cells were implanted into the brain of nude mice. One week after implantation, the tumor cells formed a mass 2 mm in diameter. From this time point, we started injection of liposomes containing human IFN-β gene 6 times every other day. One month later, the tumor was eradicated completely, although repeated direct injections of human IFN-β protein (1000 IU) did not suppress the tumor growth at all. We therefore analyzed in detail the mechanisms of this surprising antitumor effect induced by IFN-β gene transfer. From our previous experiments, we speculated that the IFN-β gene has four main anti-tumor effects on glioma cells (Fig. 2). Interestingly, IFN-β gene transfer by means of cationic liposomes induces apoptosis of cultured human glioma cells that are resistant to IFN-β protein.

Fig. 3 summarizes the molecular mechanisms of apoptosis induced by IFN-β gene transfer via cationic liposomes. Susceptibility to extrinsically supplied IFN-β protein correlated closely with the amount of intracellular IFN-β mRNA in cultured human glioma cells, in agreement with the findings of Hanson *et al*. in melanoma cell lines.[19] It was also confirmed that there is a significant prolongation of phosphorylation time of several proteins involved in the intracellular signal transduction pathway of IFN-β, such as JAK1, Tyk2, and STAT-1. This apoptotic process did not involve caspase-3 or 8 activation and cleavage of DFF45/ICAD, but activation of caspase-7 and DNase γ was detected.[20] Besides the activation of DNase γ, the interaction of cell membranes and lipid membranes on cationic liposomes in IFN-β protein-resistant cells was also required (Fig. 3). Accordingly, cell death induced by the IFN-β gene delivered in cationic liposomes probably involves at least two elements, i.e., the intrinsic apoptotic pathway and mitotic catastrophe. The former is a pathway triggered by various extracellular and intracellular stresses, such as hypoxia and DNA damage. The stress signals converge mainly on mitochondria, forming the apoptosome which contains cytochrome *c*, apoptotic protease activating factor-1 (Apaf-1) and caspase 9, and

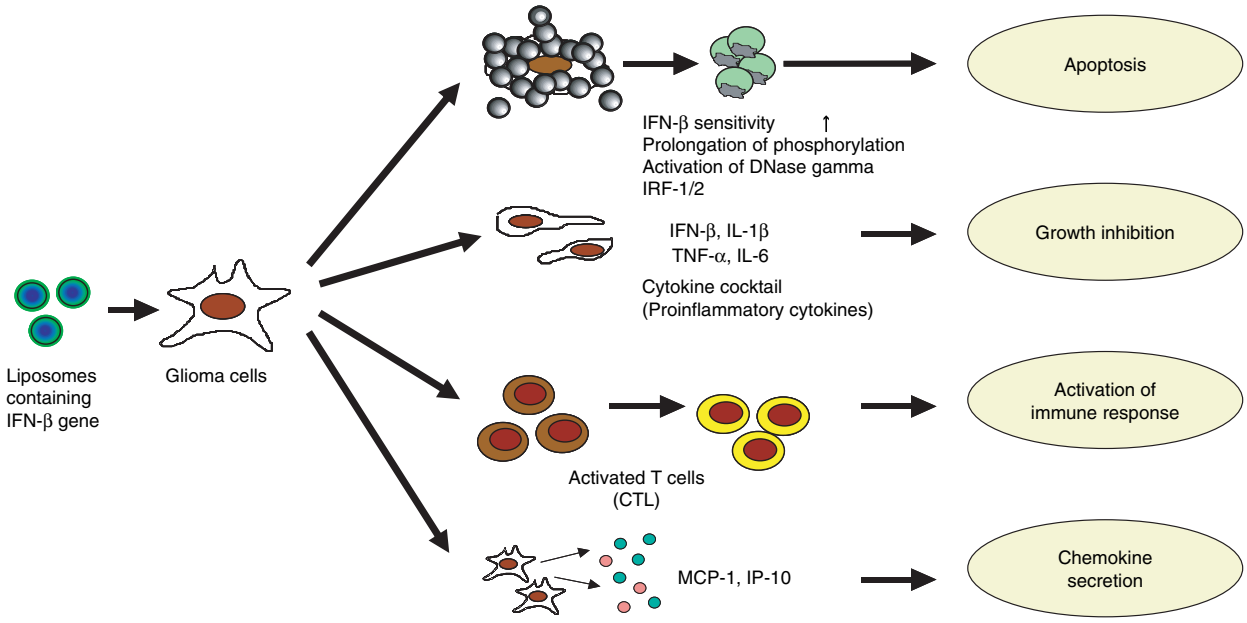


Fig. 2. Summary of antitumor mechanisms of IFN-β gene therapy for malignant glioma. There are at least four antitumor mechanisms of IFN-β gene therapy for malignant glioma; induction of apoptosis. IFN-β gene transfer by means of cationic liposomes can induce apoptosis in IFN-β protein-resistant cells; IFN-β gene transfer to glioma cells produces some cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in addition to IFN-β. The mixture of these cytokines exerted a strong antitumor effect on glioma cells; IFN-β gene transfer activates systemic immune responses and facilitates immune cell infiltration into a brain tumor, although the brain is an immunologically privileged site. Tumor-infiltrating cells are mainly CD8-positive cytotoxic T lymphocytes (CTLs) and macrophages; IFN-β gene transfer to glioma cells also produces some chemotactic factors such as monocyte chemotactic protein (MCP)-1 and IP-10.

leading to activation of caspase-7, followed by activation of DNase γ. The latter results in mitotic catastrophe, a pathway triggering mammalian cell death through aberrant mitosis. In fact, IFN-β gene transfer by means of cationic liposomes increased the number of multinucleated giant cells, i.e., the rate of abnormality of chromosome segregation, and subsequently induced apoptosis in cultured human glioma cells. Accordingly, we speculate that IFN-β gene transfer could be relevant to mitotic catastrophe in human glioma cells.

In addition to apoptosis, glioma cells transduced by the IFN-β gene produced interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, IFN-γ-inducible protein-10 (IP-10), and heat shock protein (HSP) in addition to IFN-β. The mixture of these cytokines exerted a strong antitumor effect on glioma cells. Duguay *et al*. reported similar results, namely that IFN regulatory factor (IRF)-3 gene transfer, which has been shown to activate type I IFN genes, can mediate important antitumor responses, increasing the inducibility of mRNAs for cytokines such as IFN-β, TNF-α, IL-6, MIP-1α, RANTES, and IP-10.[21] Moreover, IFN-β gene transfer activated systemic immune responses and facilitated immune cell infiltration of the brain tumor, despite the fact that the brain is an immunologically privileged site. Tumor infiltrating cells were mainly CD8-positive cytotoxic T lymphocytes (CTLs) and macrophages. According to Zhang *et al*., IFN-β gene therapy for human prostate cancer stimulated expression of inducible nitric oxide synthase, down-regulated transforming growth factor (TGF)-β and IL-8, reduced microvessel density, and resulted in apoptosis of endothelial cells in the lesions. These data suggest that macrophages may play an important role in IFN-β gene therapy.[22] Additionally, we found that dendritic cells (DCs) or macrophages which had been injected into the brain moved to the cervical lymph node. This finding suggests that macrophages may transport antigen information to the cervical lymph node. We also found that subcutaneous injection into the neck of plasmacytoid DCs, which are CD11c(+) and B220(+), effectively promoted the infiltration of CTLs in a mouse experimental glioma, while subcutaneous injection into the neck of myeloid DCs, which are CD11c(+) and B220(−), only slightly promoted it. From these findings, we speculate that there may be an "immune circuit" for brain tumors, which can be activated by the IFN-β gene delivered in cationic liposomes, as shown in Fig. 4.

2. Pre-clinical studies

In pre-clinical studies, we investigated how to make cationic liposomes containing IFN-β gene for clinical use and also how to assess safety. We have conducted careful, repeated safety tests on the liposomal products (Table 1). We did not encounter any problems in animal studies using several species, including mice, rats, dogs, and monkeys. Animal studies revealed that cationic liposomes containing the IFN-β gene are most effective when administered by a regimen that will maintain a constant low concentration of IFN-β protein for a definite time in treated tumors, rather than a single bolus administration.

We have developed freeze-dried (lyophilized) cationic liposomes, which retain therapeutic activity for more than 1 year. The lyophilization technique has made it possible to transport this gene drug (cationic liposomes containing IFN-β gene) to other institutes collaborating in this work. In 2004, we sent our gene drug to Shinshu University Hospital to begin clinical research on IFN-β gene therapy for malignant melanoma.

3. Clinical studies

3-1 Regulation of gene therapy (Fig. 5)

The regulation of gene therapy is intended to ensure not only proper assessment of risks, but also a suitable safety margin. Currently, any clinical trial should follow Good Clinical Practice (GCP) in compliance with worldwide consolidated GCP guidelines.

In our hospital, a gene therapy committee was formed prior to our clinical study. As the first step, we selected candidates

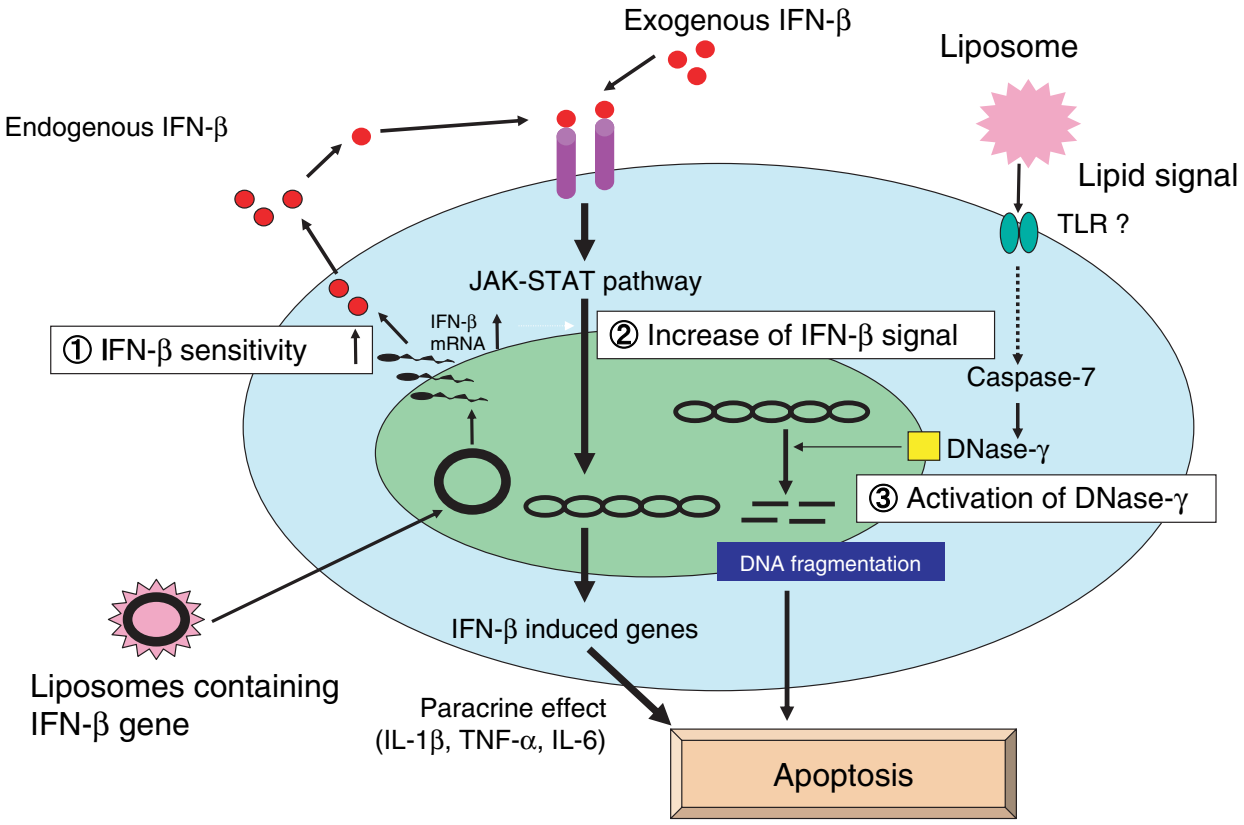


Fig. 3. Molecular mechanisms of apoptosis induced by IFN-β gene transfer by means of cationic liposomes. There are at least three molecular pathways of apoptosis induced by IFN-β gene transfer by means of cationic liposomes; the susceptibility to extrinsically supplied IFN-β protein correlates closely with the amount of intracellular IFN-β mRNA in cultured human glioma cells; IFN-β gene transfer causes a significant prolongation of phosphorylation time of several proteins involved in the intracellular signal transduction pathway of IFN-β, such as JAK1, Tyk2, and STAT-1; IFN-β gene transfer activates DNase γ through the interaction of cell membrane and lipid membrane on cationic liposomes even in IFN-β protein-resistant cells.

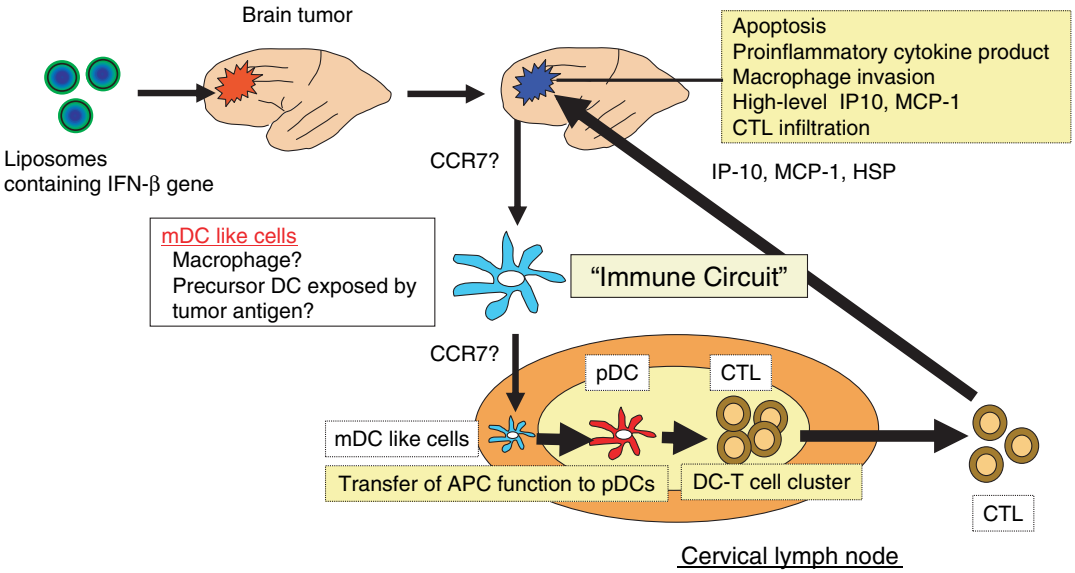


Fig. 4. Hypothetical pathway of cytotoxic T lymphocyte (CTL) activation in the brain induced by IFN-β gene therapy. We speculate that macrophages or plasmacytoid DCs (pDCs) probably transport antigen information to cervical lymph nodes, forming DC-T cell clusters, and then producing activated CTLs. The CTLs are guided to the brain tumor by IP-10, MCP-1, and HSP, then attack the tumor cells. mDC, myeloid dendritic cells; CCR, C-C chemokine receptor; APC, antigen-presenting cells; IP-10, IFN-γ-inducible protein-10; MCP-1, monocyte chemotactic protein-1; HSP, heat shock protein.

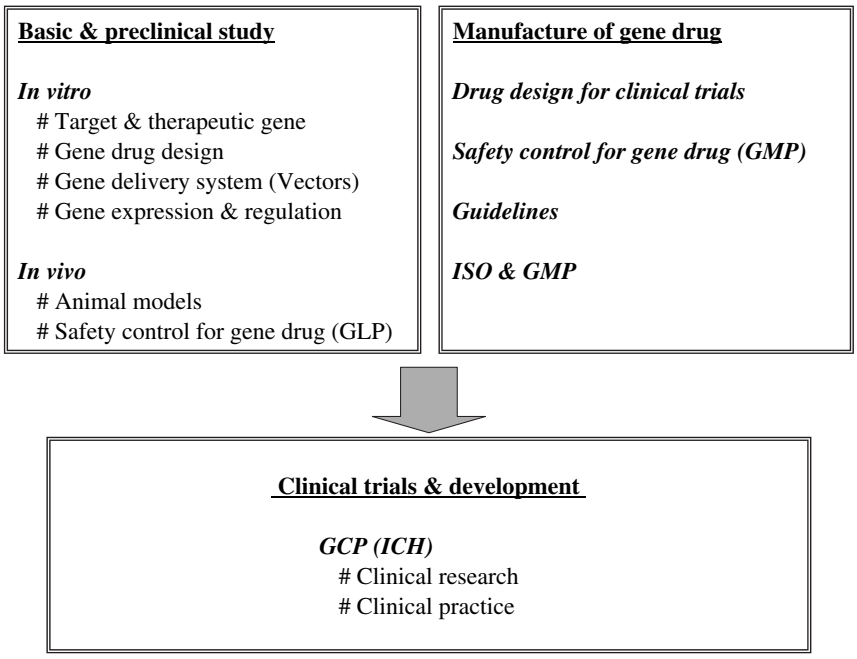


Fig. 5. Regulation and implementation of gene therapy. GLP, good laboratory practice; GMP, good manufacturing practice; ISO, international organization for standardization; ICH, international conference on harmonization.

Table 1. Safety control for gene drug (liposomes containing human interferon-β gene)

1. Single injection toxicity test (Intracranial and intravenous injection in rats and monkeys)
2. Repeated injection toxicity test (30-day repeat injection toxicity test for rats and monkeys)
3. Reproductive toxicity test
4. Deformity test
5. Antibody measurement
6. Toxicokinetics
7. Test for fever-producing activity in rabbits

No problems were detected in animal studies in several species including mice, rats, dogs, and monkeys.

who met the criteria for our gene therapy. Next, a subcommittee composed of several medical doctors, called the subcommittee for judging safety, efficacy, and indications, assessed the candidates using clinical data, and decided whether each patient would be a suitable candidate for our gene therapy. After a positive decision, a human gene therapy advisory board, composed of doctors, nurses, ethical specialists, legal professionals, and an outsider carefully reviewed the selection process again, to finally confirm the suitability of the candidate.

The manufacture and distribution of the gene drug were also of critical importance. Especially in the preparation of a gene drug, strict adherence to good manufacturing practice (GMP) is mandatory. In accordance with the regulations, our clinical-grade gene drug was produced in the Human Gene Therapy Vector Producing Facility at Nagoya University Hospital, where a fully documented quality management system is implemented. This system is similar to the management system of the International Organization for Standardization (ISO). In the near future, the development of advanced medicines will require ISO and GMP approval in Japan.

3-2 Clinical protocol

The patient received open surgery for tumor resection, followed by stereotactic injection of the gene drug. Treatment consists of reoperation and injection of liposomes containing human IFN-β gene on days 0, 14, 17, 21, 24, and 28 (first case) or 0, 14, 21, and 28 (other cases). The surgical margin of the cavity after tumor removal was infiltrated with 1 ml of the gene drug at a concentration of 30 μg DNA/ml, evenly distributed at multiple sites. From injections 2 to 6, the procedure was repeated stereotactically under local anesthesia. After the 28th day of treatment, patients entered a follow-up period and were evaluated 3 months after the first injection, then every 3 months through the third year, and then annually until the study was terminated at the patients' death.

The clinical end points were evaluatation of the safety of this gene drug and determination of the efficacy of this gene therapy.

3-3 Case reports

So far we have performed the therapy on five patients. A brief summary is shown in Table 2. The tumor of patient 1 had shown rapid progression before gene therapy, with the volume increasing about 13-fold in only 4 weeks (3.0 ml to 38.7 ml). After gene therapy, tumor growth ceased, as measured by outlining the enhanced area in MRI, and there was little change in size for the following 10 weeks. Growth then resumed, and the patient died approximately 3 months later. Patients 2 and 3 each had a partial response (PR). Patient 4 could not be evaluated because no viable tumor cells could be confirmed to exist in the enhanced mass, after γ-knife therapy. Patient 5 had stable disease during the 10 weeks following the first injection. The general and neurologic condition of all patients was unchanged or improved 3 months after starting therapy, except for patient 5. Before the therapy, patients had various neurological deficits such as hemiparesis (patients 1 and 5), aphasia (patients 1 and 5), and memory disturbance (patients 2 and 4). In particular, patient 1 could not walk or speak before the therapy because of severe right hemiparesis and motor aphasia. However, at her discharge from the hospital 3 months later, she could walk by herself and talk with her family. Patients 2 and 3 had long survivals of 29 and 26 months after tumor recurrence, respectively.[23)]

Transduced gene product in the fluid collected was assessed by EIA. Samples obtained from all patients before injection contained no detectable IFN-β protein. After injection, IFN-β protein was detected by EIA in fluid from the tumor bed in

cases 1, 2, and 5. The highest concentration, 23 IU/ml, was seen in patient 1 10 days after the first injection. IL-1β was detected in patients 1 and 5, and TNF-α was detected in patients 1, 2, and 5. Each protein was detected a few days after injection, reached maximum concentration 10 days later, then decreased gradually. IFN-β mRNA was also detected in tumor-rich tissues (patients 1, 2, 3, and 5) obtained by microdissection and examined by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). In contrast, mRNA for TNF-α was detected in tissues containing many macrophages (patients 1 and 2), but not in tumor-rich tissues. In addition, we confirmed the dramatic induction of immune response in the treated tumor tissues. After therapy, tumor tissues showed dramatic changes in all patients. Many tumor cells showed shrinkage or picnosis of the nuclei, reflecting apoptosis or necrosis. Simultaneously, MIB-1-positive cells were notably decreased. These alterations were observed over an area a few centimeters in diameter. Immunohistochemistry identified many CD8(+) lymphocytes and macrophages infiltrating into the tumor and surrounding brain, while few CD4(+) lymphocytes or B lymphocytes were present.[23] These findings were the same as reported by Brown, i.e., the antitumor response mediated by IFN-β gene delivery relied on CD8(+) T cells, but was completely independent of CD4(+) T cells.[24] Notable cell infiltration was detected at 2 weeks after injection in all patients; infiltrates then gradually increased, persisting for at least 1 month after the first injection.

Based on these results, we compared the antitumor mechanisms observed in the basic and clinical studies. We confirmed that these were almost the same in all patients treated with IFN-β gene therapy, except patient 4 (Fig. 6).

4. Expansion of clinical indications to other malignancies

4-1 Melanoma

The incidence of malignant melanoma has been increasing by 5% per year for the last 40 years in Caucasian and other populations. Patients with this neoplasm have a poor prognosis. Unfortunately, there is no effective treatment when melanoma

Table 2. Clinical results of IFN-β gene therapy for recurrent malignant gliomas

Case	Gene therapy (pDRSV-IFN-β)	Results			
		Tumor size (MRI) (3 months after Tx)	Histology	TTP (month)	D (month)
1	30 μg×1 15 μg×5	Stable/progression	Tumor cell death Immune response	3	D (6)
2	30 μg×4	Partial response	Tumor cell death Immune response	16	D (29)
3	30 μg×1	Partial response	Tumor cell death Immune response	15	D (26)
4	30 μg×2	Stable	Tumor cell death Immune response	6	D (13)
5	30 μg×4	Stable	Tumor cell death Immune response	5	D (11)

TTP, time to pregression; D, dead; Tx, treatment.

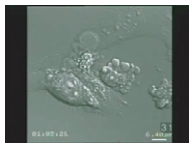
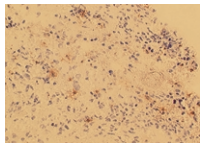
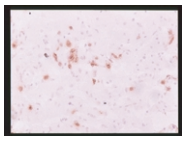
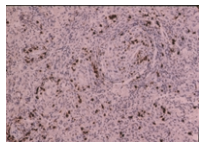
Mechanisms	Basic Study	Clinical Study
Apoptosis	 Apoptotic cells ↑	 ApopTag-positive cells ↑
Cytokine mixture	IFN-β, IL-1β, IL-6, TNF-α	IFN-β, IL-1β, IL-6, TNF-α
CTL induction	 CD8-positive cells in GL261 glioma ↑	 CD8-positive cells in patient glioma ↑
Chemotactic factors & other	MCP-1, IP-10, HSP	MCP-1, IP-10

Fig. 6. Comparison of antitumor mechanisms of IFN-β gene therapy found in basic and clinical studies. Basic studies on the antitumor mechanisms of IFN-β gene therapy were consistent with clinical findings. HSP, heat shock protein.

is recurrent and/or at an advanced stage. Gene therapy has therefore received particular attention as a promising treatment modality for melanoma. Many investigators have performed new strategic gene therapies including IL-2, human leucocyte antigen (HLA)-B7β2m, granulocyte-macrophage colony-stimulating factor (GM-CSF), gp100, and MART-1. Recently, cationic liposomes have been used as a safer alternative to virus vectors in experimental and/or clinical trials on melanoma.[25, 26] We also confirmed that melanoma is susceptible to IFN-β protein, like glioma, and we assessed the growth-inhibitory effect of IFN-β gene transferred in cationic liposomes in *in vitro* and *in vivo* experiments. As expected, cationic liposome-mediated IFN-β gene therapy was effective against melanoma, inducing direct cell death and stimulating the host immune system.[27, 28] Thus, in experiments using an experimental human melanoma implanted subcutaneously in nude mice, we found extensive apoptotic tissue and a significant decrease of Ki67-positive cells after IFN-β gene transfer.[27] In experiments using subcutaneous mouse melanoma in immunocompetent mice, liposomes containing the murine IFN-β gene, but not recombinant murine IFN-β, induced dramatic apoptosis, including nuclear condensation, shrinkage of cells, bleb formation, and ballooning. Immunocytochemical analysis demonstrated that a larger number of natural killer cells infiltrated the tumor following the gene treatment as compared with the controls. *In vivo* depletion of NK cells using anti-asialoGM1 antibody reduced the efficacy of liposomes containing the murine IFN-β gene treatment. Taken together, our data demonstrated that cationic liposome-mediated IFN-β gene therapy could be effective against melanoma by inducing direct cell death and by stimulating NK cells.[28] In this experiment, we initially expected that IFN-β gene transfer would activate CTLs in melanoma as well as glioma. However, activated immune cells were not CTLs, but NK cells. Thereafter, it became clear that major histocompatibility complex (MHC) class I expression was small in the murine melanoma model (B16F1) used in this experiment. Moreover, we found that combined therapy of cationic liposome-mediated murine IFN-β gene therapy and DCs effectively induced CTLs because a co-culture of murine melanoma (B16F1), DCs, and naïve T lymphocytes produced large amounts of IFN-β and IL-12, and IFN-β increased MHC class I expression on the surface of the melanoma cells.

In any case, liposomes containing the murine IFN-β gene played an important role in activating immune responses. We found that DCs pulsed with tumor extract-cationic liposomes complex increased the induction of CTLs in mouse brain tumor.[29]

4-2 Renal cell carcinoma

We examined the feasibility of IFN-β gene therapy for renal cell carcinoma and confirmed that it was effective. Cationic liposomes containing IFN-β gene significantly induced apoptosis, although recombinant human IFN-β protein failed to do so, suggesting clinical applicability of gene therapy for renal cell carcinoma.[30]

5. Future directions

The success of gene therapy for cancer depends on a combination of applied bioengineering and so-called translational research. In the development of previous gene therapies for cancer, the biological principles were sound, but it proved difficult or impossible to translate these principles into reality. Although suitable systems with potential for cancer gene therapy have been known for a long time, efforts have generally remained at the preclinical stage, especially in Japan. In other countries, herpes simplex virus-thymidine kinase (HSV-tk)-based suicide gene therapy has just completed phase III trials. Therefore, our home-produced technology for IFN-β gene therapy is an important step forward.

Although the search for new vectors (viral and non-viral) continues, cationic liposomes are among the most fascinating vectors for cancer gene therapy because they are non-infective, have low immunogenicity, low toxicity, and high stability, and are not expensive to manufacture. The cost-benefit relationship is important, especially in the development of advanced medicines. Moreover, this protocol needs to be combined with other advanced medicines. Indeed, lessons learnt in developmental studies of cancer gene therapy may contribute to the development of other advanced medicines, such as molecular targeting therapy, regenerative medicine, cell therapy, and organ transplantation. The understanding of antitumor mechanisms in cancer gene therapy helps us to identify candidate target molecules for molecular targeting therapy and also leads to new approaches, such as the combination of gene therapy and chemotherapy or antibody therapy. The former approach is exemplified by the dramatic results with STI-571 (Gleevec) in chronic myelogenous leukemia, and the latter by the use of anti-vascular endothelial growth factor (Avastin) and anti-epidermal growth factor (Cituximab) monoclonal antibodies in the management of advanced colorectal cancer. An improved understanding of antitumor mechanisms in cancer gene therapy will have many spin-offs.

1. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JR, Ringold GM, Danielson M. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 1987; **84**: 7413–4.
2. Felgner RL, Ringold GM. Cationic liposome-mediated transfection. *Nature* 1989; **337**: 387–8.
3. Mizuno M, Yoshida J, Sugita K, Inoue I, Seo H, Hayashi Y, Koshizaka T, Yagi K. Growth inhibition of glioma cells transfected with the human β-interferon gene by liposomes coupled with a monoclonal antibody. *Cancer Res* 1990; **50**: 7826–9.
4. Mizuno M, Yoshida J, Sugita K, Yagi K. Growth inhibition of glioma cells of different cell lines by human interferon-β produced in the cells transfected with its gene by means of liposomes. *J Clin Biochem Nutr* 1990; **9**: 73–7.
5. Yoshida J, Mizuno M, Yagi K. Secretion of human β-interferon into the cystic fluid of glioma transfected with the interferon gene. *J Clin Biochem Nutr* 1991; **11**: 123–8.
6. Yoshida J, Mizuno M, Yagi K. Antitumor effect of endogenous human β-interferon on malignant glioma and augmentation of the effect by tumor necrosis factor-α. *J Clin Biochem Nutr* 1992; **12**: 153–60.
7. Mizuno M, Yoshida J, Oyama H, Sugita K. Growth inhibition of glioma cells by liposome-mediated cell transfection with tumor necrosis factor-α gene. Its enhancement by prior β-interferon treatment. *Neurol Med Chir (Tokyo)* 1992; **32**: 873–6.
8. Yagi K, Noda H, Kurono M, Ohishi N. Efficient gene transfer with less cytotoxicity by means of cationic multilamellar liposomes. *Biochem Biophys Res Commun* 1993; **196**: 1042–8.
9. Shin N, Pardridge WM. Noninvasive gene targeting to the brain. *Proc Natl Acad Sci USA* 2000; **97**: 7567–72.
10. Mizuno M, Yoshida J. Improvement of transduction efficiency of recombinant adeno-associated virus vector by entrapment in multilamellar liposomes. *Jpn J Cancer Res* 1998; **89**: 352–4.
11. Natsume A, Mizuno M, Ryuke Y, Yoshida J. Cationic liposome conjugation to recombinant adenoviral vector reduces viral antigenicity. *Jpn J Cancer Res* 2000; **91**: 363–7.
12. Mizuno M, Ryuke Y, Yoshida J. Cationic liposomes conjugation to recombinant adenoviral vectors containing herpes simplex virus thymidine kinase gene followed by ganciclovir treatment reduces viral antigenicity and maintains antitumor activity in mouse subcutaneous glioma model. *Cancer Gene Ther* 2002; **9**: 825–9.
13. Miyakoshi J, Dobler KD, Allalunis-Turner J, McKean JDS, Petruk K, Allen PBR, Aronyk KN, Weir B, Huyser-Wierenga D, Fulton D, Urtasun RC, Day RS III. Absence of IFNA and IFNB genes from human malignant glioma cell lines and lack of correlation with cellular sensitivity to interferons. *Cancer Res* 1990; **50**: 278–83.
14. James CD, Carlbom JHE, Nordenskjold M, Cavenee WK, Collins VP. Chromosome 9 deletion mapping reveals interferon β and interferon β-1 gene deletion in human glial tumors. *Cancer Res* 1991; **51**: 1684–8.
15. Larsson I, Landstrom LE, Larner E, Lundgren E, Miorner H, Strannegard O.

Interferon production in glia and glioma cell lines. *Infect Immun* 1978; **22**: 786–9.
16. Nehashi K, Yoshida J, Wakabayashi T, Nagata M, Utsumi J, Naruse N, Sugita K. Growth inhibition of human glioma cells by superinduced human interferon-β. *Neurol Med Chir (Tokyo)* 1995; **35**: 719–22.
17. Salazar AM, Levy HB, Ondra S, Kende M, Scherokman B, Brown D, Mena H, Martin N, Schwab K, Donovan D, Dougherty D, Pulliam M, Ippolito M, Graves M, Brown H, Ommaya A. Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose; an open pilot study. *Neurosurgery* 1996; **38**: 1096–104.
18. Kaynor C, Xin M, Wakefield J, Barsoum J, Qin XQ. Direct evidence that IFN-beta functions as a tumor-suppression protein. *J Interferon Cytokine Res* 2002; **22**: 1089–98.
19. Hanson C, Koepf I, Weijdegard A, Stierner U. Sensitivity to extrinsically supplied interferon and the endogenous expression of interferon in melanoma cell lines. *Melanoma Res* 1999; **9**: 451–6.
20. Saito R, Mizuno M, Kumabe T, Yoshimoto T, Tanuma S, Yoshida J. Apoptotic DNA endonuclease (DNase-γ) gene transfer induces cell death accompanying DNA fragmentation in human glioma cells. *J Neuro-Oncol* 2004; **67**: 273–80.
21. Duguay D, Mercier F, Stagg J, Martineau D, Bramson J, Servant M, Lin R, Galipeau J, Hiscott J. *In vivo* interferon regulatory factor 3 tumor suppressor activity in B16 melanoma tumors. *Cancer Res* 2002; **62**: 5148–52.
22. Zhang F, Lu W, Dong Z. Tumor-infiltrating macrophages are involved in suppressing growth and metastasis of human prostate cancer cells by IFN-β gene therapy in nude mice. *Clin Cancer Res* 2002; **8**: 2942–51.
23. Yoshida J, Mizuno M, Yoshida J, Mizuno M, Fujii M, Kajita Y, Nakahara N, Hatano M, Saito R, Nobayashi M, Wakabayashi T. Human gene therapy for malignant gliomas (glioblastoma multiforme and anaplastic astrocytoma) by *in vivo* transduction with human β-interferon gene using cationic liposomes. *Hum Gene Ther* 2004; **15**: 77–86.
24. Brown JL, Barsoum J, Qin XQ. CD4+ T helper cell-independent antitumor response mediated by murine IFN-β gene delivery in immunocompetent mice. *J Interferon Cytokine Res* 2002; **22**: 719–28.
25. Parmiani G, Colombo MP. Somatic gene therapy of human melanoma: preclinical studies and early clinical trials. *Melanoma Res* 1995; **5**: 295–301.
26. Nabel GJ, Gordon D, Bishop DK, Nickoloff BJ, Yang Z-Y, Aruga A, Cameron MJ, Nabel EG, Chang AE. Immune response in human melanoma after transfer of an allogeneic class I major histocompatibility complex gene with DNA-liposome complexes. *Proc Natl Acad Sci USA* 1996; **93**: 15388–93.
27. Kageshida T, Mizuno M, Ono T, Matsumoto K, Saida T, Yoshida J. Growth inhibition of human malignant melanoma transfected with the human interferon-β gene by means of cationic liposomes. *Melanoma Res* 2001; **11**: 337–42.
28. Ryuke Y, Mizuno M, Natsume A, Suzuki O, Nobayashi M, Kageshita T, Matsumoto K, Saida T, Yoshida J. Growth inhibition of subcutaneous mouse melanoma and induction of natural killer cells by liposome-mediated interferon-β gene therapy. *Melanoma Res* 2003; **13**: 349–56.
29. Aoki H, Mizuno M, Natsume A, Tsugawa T, Tsujimura K, Takahashi T, Yoshida J. Dendritic cells pulsed with tumor extract-cationic liposomes complex increase the induction of cytotoxic T lymphocytes in mouse brain tumor. *Cancer Immunol Immunother* 2002; **50**: 463–8.
30. Nakanishi H, Mizutani Y, Kawauchi A, Ukimura O, Shiraishi T, Hatano M, Mizuno M, Yoshida J, Miki T. Significant antitumoral activity of cationic multilamellar liposomes containing human IFN-β gene against human renal cell carcinoma. *Clin Cancer Res* 2003; **9**: 1129–35.