Gene Therapy of Brain and Endocrine Tumors

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Gene therapy of cancer has become a major interest of medical research since more than 60% of the ongoing gene therapy protocols today involve cancer patients. To increase the therapeutic index of cancer gene therapy, targeting strategies have been developed to ensure that the expression of therapeutic genes is restricted exclusively to the tissue of interest. An attractive approach lies in the possibility to control the expression of therapeutic genes at the transcriptional level by the introduction of tissue-specific or tumor-specific enhancers/promoters offers. We have developed transcriptionally targeted vectors for gene therapy of solid tumors, including malignant gliomas and epithelial thyroid tumors. The choice of these tumor types relies on their clinical impact, ie, morbidity and mortality, the lack of effective conventional therapeutic strategies, and the ability of these tumors to express tissue/tumor-specific genes, whose transcriptional control elements (enhancer/promoter) may be used for achieving selective transgene expression. Here we report our clinical and preclinical experience in gene therapy of brain and thyroid tumors, and review the literature published on this topic.

Key words: gene targeting; gene therapy; genetic vectors; glioblastoma; thyroid neoplasms

Since more than 60% of the ongoing gene therapy protocols today involve cancer patients, gene therapy of cancer has become a major interest of many researchers. Gene therapy, as a new strategy targeted to fundamental processes of malignant cell biology, proves to be of help in fighting tumors when applied in combination with conventional cancer treatments (surgery, chemotherapy, and radiotherapy). However, it is clear from the results of the first clinical trials of gene therapy in tumor patients that the efficiency of gene delivery systems has to be improved. In this respect, when viral vectors are used, titer and tumor targeting become the critical aspects to be considered.

The issue of gene targeting vectors in cancer therapy is inextricably linked to their titer. If greater amount of available vector ends up in therapeutically irrelevant cells in vivo, the effective titer is reduced relative to its full potential. In addition, transduction of non-tumor cells is likely to be either neutral at best, or toxic at worst to the patient. Therefore, research is currently focused on redirecting the tropism of pre-existing vectors to desired cell types. Efforts have been primarily directed at manipulating cell surface-binding and transcriptional specificity (transcriptional targeting), and some results have been encouraging, both in vitro and in vivo.

In an attempt to increase the therapeutic index of cancer gene therapy, targeting strategies have been developed to ensure that expression of therapeutic genes is restricted exclusively to the tissue of interest. This is particularly important for strategies using suicide genes, in which a low-level expression of toxic genes in normal tissues may lead to severe toxicity (1). Targeted gene therapy can be obtained in many ways. The easiest one is to administer the vector directly to the target site. However, for systemic administration molecular engineering is required to target either gene expression or gene delivery. An attractive approach lies in the possibility of controlling the expression of therapeutic genes at the transcriptional level by the introduction of tissue-specific or tumor-specific enhancers/promoters.

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Glioblastoma Multiforme

Glioblastoma multiforme (GBM) represents 15-20% of all intracranial tumors and 50% of gliomas. It affects 5,000 Americans and 1,000 Italians every year, occurring typically in adults, with a peak incidence in the fifth and sixth decades of life. GBM is a very aggressive tumor, with a uniform and profound morbidity. It contributes to the cost of cancer treatment per patient more than any other tumor. The
prognosis of GBM remains extremely poor and has not changed over the last two decades, despite the combined therapy (surgery, radiotherapy, and chemotherapy). Death is caused by tumor recurrence in 80% of patients, 6-12 months after the first treatment.

GBM (grade IV astrocytoma) is usually located in the cerebral hemispheres, though it occasionally appears at other sites, such as the cerebellum, brain stem, and spinal cord. Histologically, GBM shows striking cytological diversity, ranging from tumors composed of small cells with scant cytoplasm to those composed of multinucleated giant cells. The World Health Organization (WHO) classification recognizes two distinct subvariants of the tumor: 1) giant cell glioblastoma, characterized by a predominance of enormous, multinucleated giant cells and, on occasion, an abundant stromal reticulin network; and 2) gliosarcoma, in which hyperplastic vascular elements have undergone sarcomatous transformation.

Surgical removal of the tumor mass, which is mandatory for precise diagnosis, improves the prognosis, although the infiltrative behavior of malignant gliomas precludes their complete resection, and in 90% of cases tumor recurs within 2 cm from the primary site. Postoperative radiotherapy is therefore commonly administered and improves survival. However, despite surgery and irradiation, few patients survive two years after diagnosis. Results of chemotherapy trials are disappointing due to the tumor intrinsic chemoresistance (2) and its localization within the central nervous system, which limits the penetration of drugs. Among malignant gliomas, GBM is the least responsive to medical treatment. Available protocols include both monochemotherapy and polychemotherapy regimens. Nitrosourea are leading drugs in glioma chemotherapy, with response rates varying from 10-40% when administered as single agent. Other drugs evaluated in monochemo therapy occasionally showed clinically and radiologically objective responses. Among these are vincristine, procarbazine, paclitaxel, and temozolomide. However, methodological bias present in most studies raises doubts about the validity of the results. The most commonly used polychemotherapy regimens for gliomas are a combination of procarbazine, vincristine, and CCNU (PVC) and mechloroethamine-vincristine-procarbazine (MOP). Response rates (complete or partial) of 17-37% have been reported for glioblastomas (3,4). Interesting results have been reported for ifosfamide-carboplatin-etoposide (ICE) regimen in GBM patients, although it was associated with severe hematological toxicity (5). The role of PVC as adjuvant chemotherapy is controversial and, overall, there is no clear-cut evidence that survival of glioblastoma patients is improved by chemotherapy (6).

More recently, the use of anti-tenascin monoclonal antibodies labeled with a suitable isotope (iodine-131 or yttrium-90) has been shown a promising locoregional radioimmunotherapy approach to hamper tumor regrowth (7,8).

Poor response to standard treatment and advances in elucidating the molecular biology of gliomas have led to the development of innovative therapeutic strategies, such as gene therapy aiming at inducing tumor cells chemosensitivity and/or immunogenicity by transfer of genes expressing prodrug-activating enzymes and cytokines.

Tumor cells proliferation in completely post-mitotic tissue, in an anatomical compartment well separated from the rest of the body, makes GBM a good candidate for gene therapy using retroviral vectors. Gene therapy of brain tumors in human patients by intra-tumoral injection of retroviral vectors producing cells was initiated in 1993 (9). The gene transferred was the herpes simplex thymidine kinase (HSV-Tk) gene, which confers sensitivity to the anti-herpes drug, ganciclovir (GCV). The treatment proved nontoxic and safe, with no evidence of systemic spread of the retroviral vector. However, clinical benefit was limited to small tumors due to low transduction efficiency.

Combined Immunomodulating and Suicide Gene Therapy of Glioblastoma Multiforme

To extend the therapeutic efficacy of gene therapy, our group has designed a new strategy by constructing a bicistronic retroviral vector that coexpresses the suicide gene (HSV-Tk) as well as the human IL-2 gene (10). After in vitro characterization of its efficacy and safety (10), the vector was successfully used in a pilot study to treat four patients with recurrent glioblastoma multiforme (11-13) (Fig. 1). In one of the patients it was possible to obtain a significant and sustained reduction (> 50% of the initial volume) of the tumor mass, which was associated with a dramatic clinical improvement. The other three patients showed areas of tumor necrosis and infiltration of immune-inflammatory cells around the site of stereotactic injection of retroviral vector producing cells. Their disease was stabilized for about a year.

Transcriptional Targeting of Gliomas

Efforts are being made toward more efficient gene transfer systems. Our group is working on the development of new generation of retroviral vectors, produced at higher titers and characterized by higher transduction efficiency, and on transcriptional targeting of therapeutic genes.

As for the latter, in ideal case vectors used for gene therapy of cancer should not reach any other cells than malignant. In other words, the expression of the therapeutic genes should be restrained to tumor cells only. Our research has focused on the transcriptional targeting of retroviral vectors by long terminal repeat (LTR) reshuffling, ie, by replacement of the viral enhancer in the LTR with target cell-specific enhancer elements.

Thus, the viral enhancer was substituted with that of the glial fibrillary acidic protein (GFAP), a protein selectively expressed in cells of glial origin.

First, we were able to demonstrate that LTR reshuffling did not influence the titer of viral particles. Tissue-specific transgene expression was then tested: glial (astrocytoma AOU373 and glioblastoma A172) cell lines, as well as control cell lines (HeLa and NIH3T3) were transduced with the vectors carrying
also shown, as the IC50 for the HeLa and NIH3T3 cells control vector. Absence of significant selectivity was where a 10-fold increase was observed for the A172 cells transduced with the control vector, whereas a 10-fold increase was observed for the AoU373 cells transduced with the targeted vector compared with theAoU373 cells transduced with control vector. Absence of significant selectivity was also shown, as the IC50 for the HeLa and NIH3T3 cells transduced with the targeted vector was only slightly higher than the IC50 for the same cell lines transduced with the control vector (Table 1).

The modified LTR and the control vector carrying an unmodified LTR. HSV-Tk expression was evaluated by a GCV sensitivity assay (MTT test) at GCV concentrations ranging from 0.01-100 µmol/L.

Table 1. Cytotoxic effect of ganciclovir in transduced glioma and control cell lines (IC50 values)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Vectors IC50 (µmol/L)</th>
<th>targeted</th>
<th>control</th>
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<tbody>
<tr>
<td>AoU373</td>
<td>70</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>A172</td>
<td>1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>NIH3T3</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

For the A172 cells transduced with the targeted vector, the IC50 value was identical to that reported for the A172 cells transduced with the control vector, whereas a 10-fold increase was observed for the AoU373 cells transduced with the targeted vector compared with the AoU373 cells transduced with control vector. Absence of significant selectivity was also shown, as the IC50 for the HeLa and NIH3T3 cells transduced with the targeted vector was only slightly higher than the IC50 for the same cell lines transduced with the control vector (Table 1).

Thyroid Carcinomas

Thyroid carcinomas represent the most common endocrine malignancy, accounting for about 1% of all human cancers. Although thyroid cancer generally responds to conventional therapy and has a relatively good prognosis, rare cases of anaplastic carcinomas and about 30% of relapses of differentiated carcinomas show an aggressive and highly malignant behavior, associated with a very poor survival. Medullary thyroid carcinomas, which represent 3-5% of thyroid cancers, are also highly aggressive and generally unresponsive to chemotherapy and radiotherapy.

Several gene therapy strategies have been designed for the treatment of thyroid carcinomas, including tumor suppressor replacement, prodrug activation, and immunomodulating gene therapy. The results of preclinical in vitro and in vivo studies of thyroid cancer gene therapy are encouraging. However, no clinical results have been published yet.

Tumor Suppressor Gene Replacement

Mutations of the TP53 tumor suppressor gene occur in about 70% anaplastic carcinomas and are related to a highly aggressive phenotype. Reintroduction of wild-type TP53 in anaplastic thyroid cell lines inhibited proliferation and restored a more differentiated phenotype (14-17). The combination of TP53 gene transduction with radiation or chemotherapy resulted in enhanced cytotoxic effects in thyroid carcinoma cell lines (16-18), even though the effectiveness of TP53-based gene therapy was limited to cells carrying an inactive TP53 (19).

Prodrug Activation

Suicide gene therapy could be a promising approach in the treatment of thyroid carcinomas. The HSV-Tk/GCV scheme was demonstrated to be effective against thyroid tumors in cell cultures in vitro and in animal models in vivo. In this respect, human follicular and anaplastic thyroid carcinoma cell lines, transduced with a retroviral vector containing HSV-Tk under the control of the cytomegalovirus promoter, were killed by GCV in a dose- and time-dependent manner. Bystander effect and radiosensitization were documented in vitro and in vivo (20). To develop thyroid-specific retroviral vectors, the bovine thyroglobulin (TG) promoter was introduced to control suicide gene expression (21). Selective killing of TG-producing cells was observed upon GCV treatment (21). Enhancement of TG promoter activity and specificity was obtained by introducing a TG enhancer upstream the TG promoter (22). Another strategy to improve thyroid-specific expression of therapeutic genes was the use of a Cre-loxP system, in which the Cre recombinase was expressed when the TG promoter was active and induced HSV-Tk expression under the transcriptional control of a strong promoter (23). A tissue-specific approach was attempted also for medullary thyroid carcinomas, using the promoter sequence of the calcitonin gene (24).

Immunomodulating Gene Therapy

Genetic immunotherapy showed interesting results in vitro and in vivo models of medullary thyroid carcinoma. In vitro infection of murine medullary thyroid carcinoma cells with an adenoviral vector harboring the mouse interleukin 2 (IL-2) gene abrogated their tumorigenicity and induced a long-lasting state of immunity in syngeneic BALB/C mice (25). In vivo intratumoral injection of the adenoviral vector resulted in the rejection and/or stabilization of established tumors in treated mice (26), without significant toxicity to other organs (27).
Transcriptional Targeting of Thyroid Carcinomas

We devised a thyroid-specific gene therapy strategy, combining tumor suicide with cytokine-promoted tumor rejection to amplify the antitumor activity. We developed a retroviral vector construct similar to that designed for malignant gliomas. Transcriptional targeting of thyroid carcinomas was obtained by replacing the viral enhancer of the α Moloney-derived (MFG) retroviral vector with the enhancer sequence of the human TG gene, yielding a chimeric LTR (MFG-TG). MFG-TG vector expressing nSLacZ as the reporter gene was initially used to evaluate chimeric LTR activity, demonstrating selective expression of the transgene in thyroid cells. To obtain the final vector, the genes encoding the prodrug-activating enzyme, HSV-Tk, and human IL-2 were inserted into MFG-TG vector (MFG-IL-2TKSN-TG). HSV-Tk and IL-2 were separated by an internal ribosome entry site and the neo selectable marker driven by the SV40 early promoter. The human packaging cell line Fly-A13, which can produce viral vectors in the form of complement-resistant particles, was transfected with vectors and selected with G418 to obtain resistant clones. Viral titers, determined by counting G418-resistant colonies of infected NIH3T3 cells, ranged between 10^5-10^7 CFU/mL. Viral supernatants were used to transduce human thyroid carcinoma cell lines (FTC-133 and WRO follicular carcinoma; and C8305, FRO, and ARO anaplastic carcinoma) and control human cell lines of non-thyroid origin. Specific expression of the transgenes in target thyroid tumor cells, demonstrated by reverse-transcriptase-polymerase chain reaction and histochemistry, was higher in follicular than in anaplastic carcinomas and enhanced by treatment with thyroid stimulating hormone.

In vitro experiments showed dose-, time-, and TG expression-dependent cell killing by transduction of MFG-IL-2TKSN-TG vector followed by ganciclovir treatment. The IC_{50} in thyroid carcinoma cells transduced with the targeted vector was similar to that in cells transduced with the non-targeted vector, whereas non-thyroid cells transduced with the targeted vector showed ganciclovir sensitivity 1,000-10,000-fold lower than those transduced with the non-targeted vector (Table 2). Experiments conducted in vivo by subcutaneous injection of transduced thyroid carcinoma and control cell lines in nude mice confirmed tissue-specificity of the targeted retroviral vector construct.

In conclusion, transcriptional targeting seems to be feasible, at least for some cell types. The replacement of the viral enhancer with a tissue-specific one in the case of thyroid tumors is able to confer selectivity of transgene expression in thyroid cells. Preferential expression of the transgenes is enhanced by thyroid stimulating hormone in vitro. The characteristics of the chosen sequence are probably crucial. Also, beside enhancer/promoter sequences, intracellular factors are important for the success of the approach and need to be better defined. Combined expression of two therapeutic genes (cytokine and suicide genes), achieved by the same vector, allow an increased anticancer effect.

### Table 2. Cytotoxic effect of ganciclovir in transduced thyroid and control cell lines (IC_{50} values)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Vector IC_{50} (µmol/L)</th>
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<tr>
<td></td>
<td>targeted</td>
</tr>
<tr>
<td>WRO</td>
<td>4.5</td>
</tr>
<tr>
<td>C8305</td>
<td>2</td>
</tr>
<tr>
<td>FTC-133</td>
<td>0.5</td>
</tr>
<tr>
<td>ARO</td>
<td>100</td>
</tr>
<tr>
<td>HeLa</td>
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References


Received: April 12, 2001
Accepted: May 28, 2001

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