Heat-inducible in vivo gene therapy of colon carcinoma by human mdr1 promoter–regulated tumor necrosis factor-α expression

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Abstract

The promoter of the human multidrug resistance gene (mdr1) harbors defined heat-responsive elements, which could be exploited for construction of heat-inducible expression vectors. To analyze the hyperthermia inducibility of the mdr1 promoter in vitro and in vivo, we used the pcDNA3-mdrp-hTNF vector construct for heat-induced tumor necrosis factor α (TNF-α) expression in transfected HCT116 human colon carcinoma cells at mRNA level by quantitative real-time reverse transcription-PCR and at protein level by TNF-α ELISA. For the in vitro studies, the pcDNA3-mdrp-hTNF-transfected tumor cells were treated with hyperthermia at 43°C for 2 h. In the animal studies, stably transfected or in vivo jet-injected tumor-bearing Ncr:nu/nu mice were treated for 60 min at 42°C to induce TNF-α expression. Both the in vitro and in vivo experiments show that hyperthermia activates the mdr1 promoter in a temperature- and time-dependent manner, leading to an up to 4-fold increase in mdr1 promoter–driven TNF-α expression at mRNA and an up to 3-fold increase at protein level. The in vivo heat-induced TNF-α expression combined with Adriamycin (8 mg/kg) treatment leads to the inhibition of tumor growth in the animals. These experiments support the idea that heat-induced mdr1 promoter–driven expression of therapeutic genes is efficient and feasible for combined cancer gene therapy approaches. [Mol Cancer Ther 2007;6(1):236–43]

Introduction

Inducible vectors are useful for the conditional expression of therapeutic genes in cancer gene therapy based on the inducibility of therapeutic gene expression by conventional cancer treatment modalities (1, 2). By this approach, the combination of conventional therapies, such as chemotherapy, radiation or hyperthermia, and gene therapy can result in considerable, additive, or synergistic improvement of therapeutic efficacy. This strategy has been successfully employed for the regulated expression of cytokine or of suicide genes (3–5).

Hyperthermia is gaining acceptance for cancer therapy and is used in therapeutic settings of treatment for, e.g., breast or colorectal carcinomas and malignant melanomas (6–9). In the clinic, local, regional, or whole-body hyperthermia is used in combination with chemotherapy and radiotherapy to enhance the efficacy of cancer treatment. Preclinical studies have shown that hyperthermia can sensitize tumor cells toward radiation and chemotherapy (10). Therefore, the combination of hyperthermia and gene therapy, in which hyperthermia mediates the expression induction of the transgene, is a promising strategy. This approach could be particularly beneficial if therapeutic genes that augment the effects of hyperthermia are expressed.

Different promoters (e.g., the promoter of the heat shock protein 70) have been extensively used in numerous studies for the heat-inducible gene expression (5, 11–15). These studies showed that hyperthermia of 39°C to 43°C can efficiently induce the transgene expression in vitro and in vivo.

The proximal promoter of the human multidrug resistance gene (mdr1) gene harbors different responsive elements, which are inducible by various stress factors (16–20). Particular heat shock elements mediate the heat-induced elevated expression of the mdr1 gene in a process of cellular stress response (21). In vitro studies have shown the binding of heat shock factor 1 to defined heat shock element motifs of the mdr1 promoter within the region from −178 to −152 and −99 to −66, suggesting that the mdr1 promoter can be employed for the construction of heat-inducible vectors (22–26).

The use of the human tumor necrosis factor α (TNF-α) gene for heat-directed expression in tumors is of interest because this cytokine is known to improve the therapeutic efficacy of hyperthermia in experimental and in clinical studies (27, 28). However, systemic cytokine treatment or sustained high-level expression by constitutive vectors is often associated with side effects of cytokine-mediated toxicity, which favors the local cytokine treatment in the tumor vicinity at moderate but therapeutically effective doses. Thus, gene therapy with cytokine gene–expressing
vectors is of particular interest to improve the therapeutic efficacy and to reduce toxicity. In this context, conditional promoters are particularly needed to mediate low basal and timely restricted but sufficiently induced transgene expression level, as, e.g., mediated by the mdr1 promoter.

In this study, we describe the evaluation of heat inducibility of the human mdr1 promoter for the expression of TNF-α in stably transfected or jet-injected human colon carcinoma cells and tumors, permitting elevated gene expression in vitro and in vivo. More importantly, combined hyperthermia-induced TNF-α expression and cytostatic drug application leads to increased cytotoxicity in transfected tumors and reduced tumor growth in vivo.

Materials and Methods

Cell Lines

The human colon carcinoma cell line HCT116 was cultured at 37°C and 5% CO₂ in RPMI 1640 (Invitrogen, Carlsbad, CA) medium, containing 10% FCS (Biochrom, Berlin, Germany; ref. 29).

Construction of the TNF-α-Expression Plasmid Vector

The 360-bp mdr1 promoter fragment (−207 to +158) was cloned into the pcDNA3 (Invitrogen) expression vector, replacing the cytomegalovirus promoter (see Fig. 1) by insertion into the NruI/XhoI sites resulting in the pcDNA3-mdrp construct (19, 20). The human TNF-α cDNA was inserted into the HindIII site of pcDNA3-mdrp, resulting in the pcDNA3-mdrp-hTNF (Fig. 1; refs. 4, 24).

![Figure 1](image_url)

**Figure 1.** Schematic representation of the cloning of the 360-bp human mdr1 promoter fragment (−207 to +153; mdr1-prom) into the pcDNA3 expression vector. This promoter fragment harbors two heat shock elements (HSE; −178 to −152 and −99 to −66) and is driving the expression of the human TNF-α gene. The heat shock element at position −178 to −152 carries the consensus tandem repeat sequence (5'-GAAAtTTC-3') known for heat shock factor 1 binding.

Establishment of Stably pcDNA3-mdrp-hTNF-Transfected Tumor Cell Clones

The pcDNA3-mdrp-hTNF vector was transfected into 1 × 10⁶ HCT116 colon carcinoma cells using lipofectin (Life Technologies) according to the manufacturer’s instructions. Forty-eight hours after transfection, vector-carrying clones were selected in G418 (0.5 mg/mL, Life Technologies)-containing medium. All isolated clones were screened for basal TNF-α expression.

**In vitro Hyperthermia Treatment of pcDNA3-mdrp-hTNF-Transfected HCT116 Cells**

In the in vitro experiments, pcDNA3-mdrp-hTNF-transfected HCT116 cells were treated with hyperthermia for 2 h at 42°C or 43°C, and controls were incubated at 37°C. For the TNF-α ELISA, 200-μL supernatants of the cells were collected at 0, 1, 2, 3, 4, 5, 12, 24, 48 h after heat shock, and at the same time, points cells were harvested for the isolation of total RNA.

**In vivo Hyperthermia Treatment of pcDNA3-mdrp-hTNF-Transfected HCT116 Tumors**

For the establishment of tumors, female Ncr:nu/nu mice were injected with 1 × 10⁷ pcDNA3-mdrp-hTNF–transfected or pcDNA3-mdrp-hTNF–nontransfected HCT116 cells into the left foot pad. At a tumor size of ~6 × 6 mm, hyperthermia experiments were started.

Hyperthermia was applied locally to the tumor for 60 min at 42°C in a temperature-controlled water bath. Before, during, and after hyperthermia, temperature of tumors was controlled. For the analysis of heat-induced TNF-α expression, animals (six animals per group) were sacrificed 0, 4, 6, 24, 48, and 72 h after hyperthermia; tumors were removed for the preparation of tumor lysates and total RNA. Serum samples were collected to evaluate the potential systemic distribution of TNF-α.

For the therapeutic in vivo experiments, tumor-bearing animals (seven animals per group) were treated once with local hyperthermia at 42°C for 60 min, followed by the chemotherapeutic treatment with 8 mg/kg Adriamycin at days 1 and 8 after hyperthermia. During the in vivo experiment, body weight and tumor size were measured to evaluate the therapeutic effect of the combined treatment.

**Analysis of In vitro or In vivo Heat-Induced TNF-α Expression by ELISA**

For the in vitro experiments, supernatants of transfected HCT116 cells were collected for the TNF-α ELISA at defined time points before or after hyperthermia. Tumor lysates from the in vivo experiments were prepared using the snap-frozen tumor tissue. Tissue samples were homogenized in 500 μL ice-cold Tris-EDTA buffer (containing 10 mg/mL aprotinin and 0.1 mg/mL phenylmethylsulfonyl fluoride) using the UltraTurrax (IKA-Labortechnik, Staufen, Germany), centrifuged at 14,000 rpm, 4°C for 10 min and subjected to ELISA. The TNF-α ELISA (Diaclone) was done according to the manufacturer’s instructions using 200 μL of cell culture supernatants in the in vitro experiments or 200 μL of tumor lysates in the in vivo experiments. Absorbance was measured in a microplate reader at 450 nm (SLT-Labinstruments, Munich, Germany; ref. 30).
Crailsheim, Germany) in quadruplicates. The TNF-α values were calculated from the respective TNF-α standard curve using the EasySoftG200/Easy-Fit software (SLT-Labinstruments). For the in vivo analyses, TNF-α values were normalized to the corresponding protein content of the tumor homogenates, determined by Coomassie Plus Protein Assay reagent (Pierce, Rockford, IL).

**Quantitative RT-PCR for Analysis of Heat-Induced TNF-α Expression**

Total RNA from cells and tissue cryosections was isolated using the TRIzol method (Invitrogen). Reverse transcriptase reaction was done with 50 ng of total RNA (MuLV Reverse Transcriptase, Perkin-Elmer, Weiterstadt, Germany). Each quantitative real-time PCR (95°C for 30 s, 45 cycles of 95°C for 10 s, 62°C for 10 s, 72°C for 10 s) was done using the LightCycler (LightCycler DNA Master Hybridization Probes kit, Roche Diagnostics, Mannheim, Germany). Expression of TNF-α and of the housekeeping gene glucose-6-phosphate dehydrogenase (G6PDH) was determined in parallel from the same reverse transcriptase reaction, each done in duplicate per sample. For TNF-α, a 144-bp amplicon (forward-primer: 5′-AGCGCTGAGATCAATCGG-3′; FITC-labeled probe: 5′-GAGGACGAACTCCAACCTTCCCA-3′-FITC; LCRed640-labeled probe: LCRed640-5′-ACGCTCCTCTGGCCTCCTCCA-3′; reverse primer: 5′-GAAGGAGGGGGTATAAAAGGG-3′), and, for G6PDH, a 113-bp amplicon were produced, which were detected by gene-specific fluorescein- and LCRed640-labeled hybridization probes (primers for TNF-α: BioTeZ, Berlin, Germany; probes for TNF-α: TIB MOLBIOL, Berlin, Germany; and primers and probes for G6PDH: Roche Diagnostics). The calibrator cDNA, derived from the human TNF-α-expressing myeloblastoma cell line HL60, was employed in serial dilutions (in duplicate) simultaneously in each run.

**Intratumoral Jet-Injection of Naked pcDNA3-mdrp-hTNF DNA and Hyperthermia Treatment**

For the nonviral in vivo gene transfer, female Ncr:nu/nu mice xenotransplanted with the nontransfected HCT116 human colon carcinoma cells were used. At a tumor size of ~6 × 6 mm, the tumor-bearing animals (two animals per time point) were anesthetized and received four intratumoral jet-injections of the pcDNA3-mdrp-hTNF plasmid DNA through the skin using the “Swiss-Injector” prototype (EMS Medical Systems SA, Nyon, Switzerland) as described (30). Each animal received a total dose of 40 μg pcDNA3-mdrp-hTNF plasmid DNA. The respective control animals were jet-injected with PBS. For analysis of TNF-α expression, animals were sacrificed before and 4, 24, and 48 h after hyperthermia, and tumors were removed and snap frozen in liquid nitrogen. Ten to twenty cryosections (15 μm) were collected, and tumor protein lysates or tumor RNA was prepared for the analyses.

In the therapeutic experiments, the jet-injected animals (eight animals per group) were treated 48 h after jet-injection.
with hyperthermia and Adriamycin as described for the stably transfected HCT116 tumors. Tumor volume and body weight was measured to evaluate the therapeutic effect of the combined treatment.

**Statistical Analysis**

The levels of statistical significance were evaluated by using the nonparametric Wilcoxon signed rank test. The statistical significance was set at \( P \leq 0.05 \). For *in vivo* tumor growth evaluation, the Mann–Whitney \( U \) test was used (\( P \leq 0.05 \)).

**Results**

**Heat-Induced mdr1 Promoter–Driven TNF-α Expression in HCT116 Cells**

For the determination of heat inducibility of human *mdr1* promoter–driven gene expression, we established stably pcDNA3-mdrp-hTNF–transfected HCT116 cell clones. Figure 2A shows the temperature-dependent induction rates 2 h after hyperthermia at 42°C or 43°C in two representative HCT116 clones, indicating that the heat-induced expression is elevating with increased temperatures and reaches a maximum of 2.5-fold induction 48 h after heat shock at 43°C. These two clones were used for further detailed analyses of heat-induced TNF-α expression at mRNA and at protein level. The cell clones were treated 2 h at 43°C, and time dependence of induced TNF-α expression was determined using quantitative real-time reverse transcription (RT)-PCR and ELISA. Figure 2B and C clearly shows the rapid 2.5- to 3-fold increase in mRNA expression as shortly as 1 h after hyperthermia. However, 24 h after hyperthermia TNF-α mRNA concentrations return to basal expression level. Timely delayed TNF-α protein expression starts to increase in the two clones (2- to 2.3-fold) between 5 and 48 h after heat treatment (Fig. 2D and E). These results indicated that the *mdr1* promoter is heat inducible in a time-dependent fashion in the pcDNA3 plasmid-vector context confirming the *in vitro* findings described earlier, where a murine leukemia virus–based retroviral vector construct was used (24).

**Heat-Induced TNF-α Expression in Stably Transfected HCT116 Tumors In vivo**

The results of *in vitro* heat induction of TNF-α expression in pcDNA3-mdrp-hTNF clones prompted us to determine the *in vivo* heat inducibility. The tumor-bearing animals were treated with hyperthermia for 15, 30, 45, or 60 min at 42°C to determine the optimal condition for heat-induced TNF-α expression. Figure 3A shows the TNF-α expression at mRNA level analyzed by real-time RT-PCR. As depicted, *mdr1* promoter–mediated TNF-α expression increases with prolongation of hyperthermic treatment. The best induction rate was achieved at 60 min with a 3.5-fold increase. Because this time and temperature range was well tolerated by the animals, we used these conditions (60 min, 42°C) for all other *in vivo* experiments.

For the more detailed evaluation of time course of heat-induced TNF-α expression, transfected HCT116 tumor-bearing animals were treated with hyperthermia at 42°C for 60 min, and tumors were removed for analysis immediately after heat treatment and 4, 6, 24, 48, and 72 h after hyperthermia. Figure 3B shows the result of real-time RT-PCR, where post-hyperthermia and 4 h after heat treatment, a rapid, up to 4-fold increase in TNF-α mRNA was detected (\( P = 0.004 \)). Although 6 h after hyperthermia,

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

Figure 3. **A**, *in vivo* correlation between duration of hyperthermia and induction level of TNF-α expression. pcDNA3-mdrp-hTNF–transfected HCT116 tumor–bearing animals were treated with hyperthermia (42°C; 15, 30, 45, or 60 min; \( n = 5 \) animals at each time point). The control animals did not receive hyperthermia. TNF-α expression was determined at mRNA level by real-time RT-PCR and was normalized to the expression of the *G6PDH* housekeeping gene. **Columns**, mean ratios of TNF-α and *G6PDH* mRNA expression that were determined in duplicates for each animal. Time course of *in vivo* heat induction of *mdr1* promoter–driven TNF-α expression at mRNA (B) and at protein level (C, \( n = 6 \) animals per group). pcDNA3-mdrp-hTNF–transfected HCT116 tumor–bearing animals were treated with hyperthermia (42°C, 60 min). B, at indicated times, TNF-α expression was determined at mRNA level using real-time RT-PCR (\( **, P = 0.004; *, P < 0.01 \)). TNF-α mRNA expression rates were normalized to the expression of the *G6PDH* gene; **Columns**, mean ratios of TNF-α and *G6PDH* mRNA expression that were determined in duplicates for each animal. C, TNF-α protein levels were determined by TNF-α ELISA and were normalized to the respective protein concentration; values were determined in duplicates for each animal (\( *, P = 0.01 \)).
mRNA level decreased, the mRNA concentration remained at an elevated level during the observation time of 48 h compared with the nonheated control animals \((P < 0.01)\). Almost similar to the in vitro kinetics (see Fig. 2D and E), an increase in TNF-\(\alpha\) protein level seems again timely delayed in the heated tumors with a maximum of 2-fold \((P < 0.01)\) elevation 4 h after hyperthermia (Fig. 3C). Over time however, TNF-\(\alpha\) expression returns to basal expression levels 48 to 72 h after hyperthermia \((P \geq 0.05)\).

**Hyperthermia-Induced TNF-\(\alpha\) Expression for Combined In vivo Tumor Therapy**

The results of the in vivo heat-induced TNF-\(\alpha\) expression indicate that this vector system is suited for therapeutic applications. We therefore tested the therapeutic effects of the combined heat-induced TNF-\(\alpha\) expression with chemotherapy in vivo. For this, transfected HCT116 tumor-bearing animals were heated only once for 60 min at 42°C. At days 1 and 8 after hyperthermia, Adriamycin was applied. The control groups received either no hyperthermia, hyperthermia only, or Adriamycin only. As shown in Fig. 4A, the tumor volumes increased over the entire observation time in the nonheated control group. Although not significant, in the control group, which was heated only, tumor growth was suppressed compared with the nonheated animals. More importantly, if heat-induced TNF-\(\alpha\) expression is combined with Adriamycin treatment, a reduction in tumor growth, significant up to 50%, is seen \((P = 0.031)\). The histologic evaluation of sections from tumors, which received no hyperthermia (Fig. 4B), hyperthermia only (Fig. 4C), or the combined treatment of heat-induced TNF-\(\alpha\) expression and drug (Fig. 4D), indicated different levels of necrosis: nontreated tumors showed only minor necrotic alterations of about 20% to 30%, whereas in hyperthermia-treated animals, 40% to 50% of the tumor is affected by necrosis, and in the tumors that received the combined treatment, more than 80% of the tumor tissue is necrotic. This indicates that the reduced tumor growth of the combined treatment accounts for the necrotic cell death of large portions of the tumor.

**Hyperthermia-Induced TNF-\(\alpha\) Expression for Combined In vivo Tumor Therapy in Preestablished pcDNA3-mdrp-hTNF Jet-Injected Tumors**

To test whether our hyperthermia-inducible gene therapy has also a therapeutic effect in a model of preestablished,
nontransfected HCT116 tumors, we used the nonviral intratumoral jet-injection gene transfer of the pcDNA3-mdrp-hTNF construct. In this approach, we first analyzed the heat inducibility of the vector. For this, tumors were jet-injected 48 h before hyperthermia and were then removed before and 4, 24, and 48 h after hyperthermia. TNF-α expression was analyzed at protein level by ELISA and at mRNA level by RT-PCR. Figure 5 shows the basal TNF-α expression at both protein and mRNA level, demonstrating the efficient jet-injection gene transfer into the tumor, although at a lower level compared with the stably transduced HCT116 tumors. This observation can be attributed to the fact that only a particular proportion of the jet-injected tumor tissue is transfected by jet-injection. In regard to heat inducibility, we observed an almost 2-fold increase in TNF-α protein expression 4 h after hyperthermia, which then rapidly returns to the basal expression level within 48 h (Fig. 5A). A similar time course of TNF-α mRNA expression was seen for these tumors by RT-PCR analysis (Fig. 5B).

It was of great interest whether this level of TNF-α expression after the nonviral jet-injection gene transfer will also lead to a therapeutic effect in vivo. We therefore applied naked pcDNA3-mdrp-hTNF DNA by intratumoral jet-injection and started the same treatment schedule as for the stably transduced tumors 48 h after jet-injection. The control groups received either no hyperthermia, hyperthermia only, or Adriamycin only.

Figure 6 shows the therapeutic effect on tumor growth in the different animal groups. It is evident that we were able to reproduce the significant reduction in tumor growth in the combined treatment group (P = 0.0078), which received hyperthermia and Adriamycin. Similar to the observation in the stably transduced tumor model, this growth-inhibitory effect lasted for the entire observation time of 21 days. Therefore, this therapeutic experiment is a demonstration that, also in the jet-injected tumors, heat-induced TNF-α expression and application of Adriamycin are effective to achieve significant tumor growth inhibition.

**Discussion**

One decisive issue in gene therapy is the establishment of regulatable vector systems for transcriptional targeting and for responsiveness to different external stimuli (31). Various conditional promoters have been used for therapy-inducible gene expression vectors (1). Inducing factors can be therapeutic modalities, such as chemotherapy, hyperthermia, or radiotherapy. Such inducible promoters should ideally exert low basal gene expression and mediate high-level induced expression of the desired therapeutic gene. The approach of a heat-inducible gene therapy is stimulated by a growing number of clinical studies showing the therapeutic benefit of hyperthermia for the treatment of different malignancies (6, 7, 9, 10). Recent technological developments significantly improved the precision to heat target areas at defined temperatures, which are also sufficient for heat-induced gene therapy. The majority of heat-inducible vector systems is based on the employment of the human heat shock protein 70 promoter or of derivatives of this promoter to achieve a high-level heat-induced gene expression (12, 14, 15, 32).

In our study, we employed the multidrug resistance gene mdr1 promoter, which is responsive toward heat stress mediated by heat shock elements (4, 17, 18, 20, 21, 33). The
current study extends the use of the human mdr1 promoter for a heat-inducible gene expression to combine gene therapy and hyperthermia to in vivo application. Previous comparative analyses of the full-length 2,100-bp mdr1 promoter and the 360-bp promoter variant have shown the superiority of the shorter variant regarding basal and also heat-induced transgene expression (24).

In this study, we have shown the feasibility of the heat-induced mdr1 promoter–driven TNF-α expression and of the combined action of hyperthermia and gene therapy in an animal model using stably transfected HCT116 tumors or tumors jet-injected with the pcDNA3-mdrp-hTNF vector. The in vitro and in vivo experiments revealed that TNF-α expression is inducible at 42°C or 43°C, with induction rates of 2- to 4-fold at mRNA and at protein level. The magnitude of the heat-induced TNF-α expression is comparable to those observed by Miyazaki et al. for mdr1 promoter activities determined in reporter studies or for endogenous mdr1/P-glycoprotein expression after heat shock at 43°C or 45°C (16, 22). Although the heat shock protein 70 promoter was shown to permit induction rates of up to several hundred-fold in response to hyperthermia of 40°C to 45°C, we have shown that in the light of a combined hyperthermia, gene therapy, and chemotherapy, the mdr1 promoter–mediated increase of TNF-α expression is sufficient for improved tumor growth inhibition in the two experimental settings of the stably transfected or the jet-injected tumors (12, 15, 24). A similar observation has been reported by the use of the growth arrest and DNA damage gene (gadd153) promoter which permitted a 3-fold induced TNF-α expression after hyperthermia at 46°C leading to a significant growth arrest in vivo (34). Therefore, the observed 2- to 3-fold increase in TNF-α expression after a single local hyperthermia of 42°C in both experimental settings in vivo is sufficient to generate therapeutic benefit. We suggest that not only the magnitude but also the duration of heat-induced transgene expression is important for the desired therapeutic effect. On the other hand, leakage of constitutive or induced artificially high-level cytokine expression into the systemic circulation can lead to unwanted serious side effects or might provoke the development of resistance toward TNF-α (35, 36). In fact, when analyzing the serum TNF-α level of animals after hyperthermia, we did not detect the systemic release of the cytokine nor associated side effects such as cachexia or body weight loss, changes in body temperature, or alterations in the content of WBC or thrombocytes (data not shown). By contrast, sustained high-level constitutive TNF-α expression can provoke these side effects by systemic leakiness and might therefore interfere with possible clinical applications.

Numerous studies have shown that the combination of hyperthermia, TNF-α, and cytostatic drugs, such as melphalan, cisplatin, or doxorubicin, can be of therapeutic benefit (27, 28, 37–39). Hyperthermia and TNF-α are able to sensitize tumors toward cytostatic drugs by different cellular mechanisms, such as activation of apoptosis, improved drug uptake, and/or reduction of drug resistance in association with increased cytotoxicity. This prompted us to investigate the effects of localized heat-induced TNF-α expression on Adriamycin cytotoxicity in the in vivo colon carcinoma model. We observed that the hyperthermia-induced increase of TNF-α expression per se did not significantly affect the tumor growth in stably transfected or in jet-injected tumors. This points to the fact that only the combination of hyperthermia-induced TNF-α expression and a cytostatic drug such as Adriamycin can lead to improved therapeutic efficacy.

In summary, we were able to show the in vivo effectiveness of our heat-induced gene therapy approach in the model of stably transfected tumors and, more importantly, in preestablished tumors nonvirotransfected by intratumoral jet-injection. Such therapeutic approach holds promise to be clinically applicable not only for the treatment of colon carcinomas but also for the therapy of, e.g., mammary, cervical, prostate, or bladder carcinoma, where regional hyperthermia could be combined with heat-regulatable gene therapy for improved antitumor efficacy.

Acknowledgments

We thank M. Lemm and L. Malcherek for excellent technical assistance and W. Haensch for the histopathologic evaluation of the tumor sections.

References


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