Chemoinducible gene therapy

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Chemoinducible cancer gene therapy is a potential new treatment for solid tumors that may in part enhance the anti-tumor effects of chemotherapy while minimizing toxicity. This approach combines viral vectors expressing cytotoxic transgenes that can be transcriptionally activated by DNA-damaging agents. The development of chemoinducible gene therapy has numerous implications for the treatment of both localized and metastatic disease in patients with solid tumors. *Anti-Cancer Drugs* 16: 1053–1058 © 2005 Lippincott Williams & Wilkins.

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Introduction

Cancer gene therapy represents a novel treatment strategy for solid tumors. One example of this therapeutic modality is the transduction of cancer cells with viral vectors that express cytotoxic cytokines [1,2]. The local delivery of these viral vectors is followed by a DNA-damaging agent that 'activates' transgene expression within the tumor stroma [3,4]. This approach, which results in localized production of cytokines following promoter activation by DNA-damaging agents, can increase the therapeutic effects of both the cytokine and the genotoxic agent. This review highlights the development of ionizing radiation (IR) as a paradigm for local gene therapy using inducible adenoviral vector delivery vehicles and how this approach evolved into chemoinducible gene therapy for cancer.

Transcriptional targeting of gene therapy by IR

An idealized model for transcriptional targeting includes tumor-specific delivery of a cytotoxic/anti-tumor transgene followed by transgene activation in association with an effective anti-tumor treatment. Tumor necrosis factor (TNF)- α is a cytokine that is being exploited for cancer gene therapy. TNF-α exerts a direct cytotoxic effect on certain tumor cells as well as tumor endothelium [5,6] and is also a known radiosensitizer under specific conditions [7]. As TNF-α confers significant systemic toxicity, localized TNF-α transgene expression induced by DNA-damaging agents provides a potential strategy for tumor therapy [8,9].

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The use of IR with adenoviral vectors has provided a paradigm for the development of gene therapy using DNA-damaging chemotherapeutic agents [3,10]. Our laboratories first reported that IR activates transcription of the early growth response-1 (Egr-1) gene via specific CArG elements within the gene's promoter region [11]. Cloning of these CArG elements upstream of a human TNF-α cDNA resulted in radiation-induced control of TNF-α production. These studies led to the development of Ad.Egr-TNF, an E1, E3-deleted, replication-defective type 5 adenovirus carrying a chimeric construct including the inducible CArG sequences of the Egr-1 promoter cloned upstream of a human TNF-α cDNA [3,5,6,12]. Once the virus is injected intratumorally, exposure to IR generates radical oxygen intermediates (ROIs), which activate the Egr-1 promoter and thereby results in localized transcription of the TNF-α gene. Localized production of TNF-α confers anti-tumor activity without systemic side-effects. The combination of Ad.Egr-TNF and IR has been effective in several preclinical models of radioresistant tumors including head and neck cancer, prostate cancer, and glioma [3,6,12]. This combination therapy is also currently being studied in clinical trials for the treatment of a variety of solid tumors [13,14].

Chemoinducible gene therapy utilizing the Egr-1 promoter (Ad.Egr-TNF)

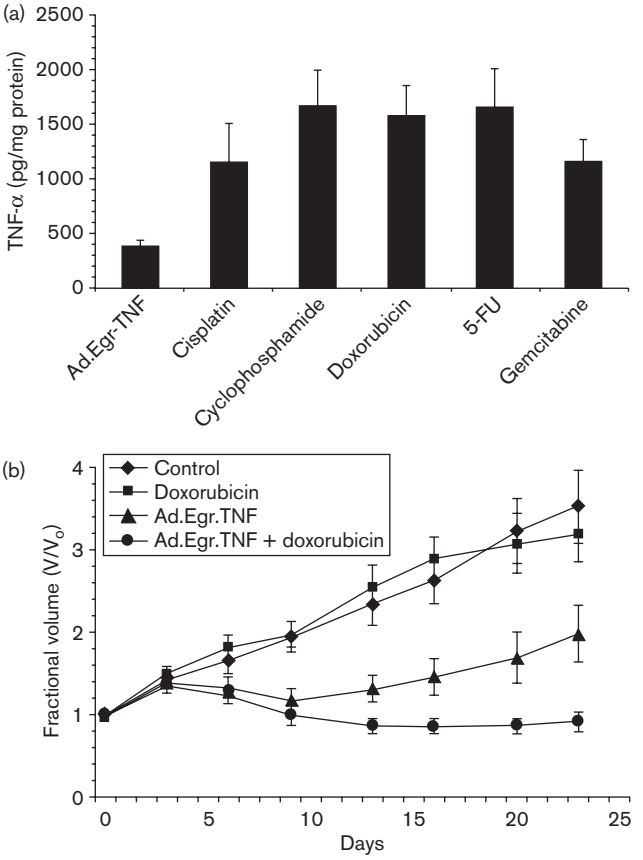
Datta *et al.* first performed deletion analysis of the Egr-1 promoter and demonstrated that a specific motif within the 5′ region [CC(A + T-rich$_6$GG], the CArG sequence, mediates the Egr-1 transcriptional response to IR and

H_2O_2 (DNA damage-induced ROIs) [11]. These experiments led to additional studies employing genotoxic anticancer drugs in combination with Ad.Egr-TNF [15,16]. Park *et al.* investigated the use of cisplatin, a known inducer of intracellular ROIs, to activate Ad.Egr-TNF [17]. The experiments were performed using a human esophageal adenocarcinoma cell line (Seg-1) and a rat colon adenocarcinoma cell line (DHD/Prob/K12). Studies *in vitro* and *in vivo* demonstrated that exposure of Ad.Egr-TNF-infected cells to cisplatin resulted in significant increases in TNF-α production compared with controls. Promoter deletion studies demonstrated that cisplatin-induced TNF-α production was due to activation of the CArG elements of the Egr-1 promoter. These results indicated that, like IR, cisplatin induces signals that target the CArG elements and that DNA-damaging agents that produce ROIs could be employed to treat solid tumors infected with Ad.Egr-TNF.

To study whether other chemotherapeutic agents known to induce ROI formation could be used to transcriptionally target Ad.Egr-TNF, Lopez *et al.* tested doxorubicin, cyclophosphamide, 5-fluorouracil (5-FU), gemcitabine and paclitaxel [18]. In these studies, human PC-3 prostate cancer cells and the Prob cells employed by Park *et al.* [17] were infected with Ad.Egr-TNF *in vitro* and exposed to each of the chemotherapeutic agents. Production of TNF-α protein as detected by ELISA was above that of controls for each agent. These results demonstrated that different classes of chemotherapeutic agents activate the Egr-TNF-α transgene. The authors also measured TNF-α transgene expression in xenografted tumors following treatment with i.p. injection of the chemotherapeutic agents. The results showed induction of the Egr-1 promoter in PC3 tumors infected with Ad.Egr-TNF (Fig. 1A). Moreover, treatment with intratumoral Ad.Egr-TNF and systemic doxorubicin resulted in a significant anti-tumor effect compared with either treatment alone in the human prostate cancer xenografts (Fig. 1B). The activation of Egr-1 transcription was suppressed by *N*-acetylcysteine, a known ROI scavenger. These results, considered with those of Datta *et al.* [11], indicated that the mechanism of Egr-1 activation was through the generation of ROIs and that transgene expression could be controlled by agents that produce ROIs. Therefore, agents that produce ROIs can be used to locally activate the Egr-1 promoter resulting in TNF-α production within the tumor tissue and cytotoxicity without systemic toxicity.

In the absence of a specifically targeted vector that has tropism for tumor and not normal tissue, the administration of systemic chemotherapy can be utilized to treat locoregional disease in combination with intratumoral gene therapy. An example of how transcriptional targeting of gene therapy by chemotherapy can be used to treat a localized cancer was demonstrated by Yamini *et al.* The

Fig. 1



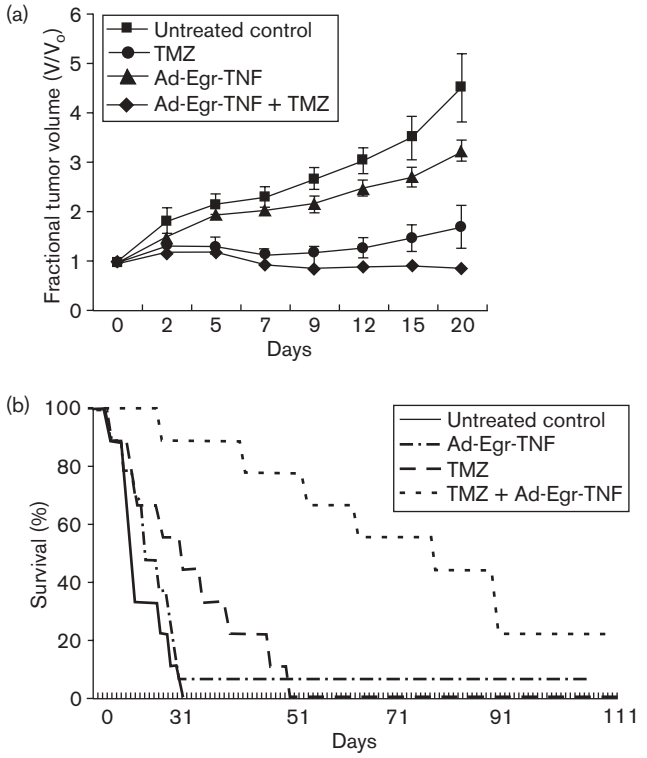
(a) Transcriptional activation of Egr-1 by chemotherapeutic agents in PC-3 tumor xenografts. PC-3 xenografts were treated by intratumoral injection of 5×10^9 p.u. Ad.Egr-TNF on days 0 and 1, and i.p. injection of cisplatin (9 mg/kg), cyclophosphamide (160 mg/kg), doxorubicin (15 mg/kg), 5-FU (100 mg/kg) or gemcitabine (500 mg/kg) on days 1 and 2. There were significant increases in TNF-α protein as detected by ELISA. (b) The combination of Ad.Egr-TNF and doxorubicin overcomes chemoresistance in PC-3 xenografts. PC-3 xenografts treated with 5×10^9 p.u. Ad.Egr-TNF on days 0 and 3 combined with 2 mg/kg of doxorubicin on days 0–8 of therapy showed a significant anti-tumor response compared to doxorubicin treatment alone. Figures adapted from [18] with permission.

investigators combined Ad.Egr-TNF with an alkylating agent, temozolomide (TMZ), in a murine model of glioblastoma [19]. As with the other chemoinduction studies, this alkylating agent activated the Egr-1 promoter through ROI formation. The combination also resulted in superior cytotoxicity compared to either treatment alone (Fig. 2A) and significantly prolonged survival (Fig. 2B). The use of this model system thus demonstrated that chemotherapy could be utilized in combination with gene therapy to treat a localized cancer.

Chemoinducible gene therapy utilizing the multidrug resistance gene (MDR1) promoter

Another well-characterized system involves exploitation of MDR1 induction. The MDR1 gene encodes a

Fig. 2



(a) Transcriptional targeting of Ad.Egr-TNF with TMZ in hind-limb tumors established from a human glioblastoma cell line. Ad.Egr-TNF was injected intratumorally twice per week (2×10^8 p.u.). Intraperitoneal TMZ (5 mg/kg) was administered 3 h following vector administration. (b) Kaplan–Meier curve demonstrating improved survival in an intracranial model of glioblastoma in mice. Animals received a single intratumoral injection of 5×10^8 p.u. of Ad.Egr-TNF followed by i.p. TMZ (5 mg/kg) 3 h later and repeat injections on days 1–4 for a total TMZ dose of 20 mg/kg. Figures adapted from [19] with permission.

transmembrane active transport protein, P-glycoprotein (P-gp), which is a drug-efflux pump that works to secrete numerous chemotherapeutic agents from cells [20]. Many tumor types such as colorectal and renal cell carcinoma are therefore intrinsically resistant to P-gp substrates secondary to their constitutive levels of MDR1 gene expression. Other tumors, initially sensitive to systemic therapy, may become chemoresistant during treatment because of upregulation of P-gp production (breast and ovarian carcinoma). Preclinical data suggests that P-gp inhibitors used in combination with cytotoxic chemotherapy may be beneficial in terms of reversal of clinical drug resistance leading to enhanced tumor regression [20].

MDR gene regulation in response to chemotherapy prompted a number of studies aimed at exploiting MDR promoter activity for targeted gene therapy. Stein *et al.* demonstrated inducibility of the MDR1 promoter in human colon carcinoma cells by the chemotherapeutic agent vincristine [21]. Two cell lines were observed to have significantly different responses with regard to MDR induction as identified by chloramphenicol acetyl transferase (CAT) assays to measure promoter induction. Reporter assays revealed a broad range, dose-dependent induction of MDR1 that was 3-fold greater in the more resistant cell types. The authors were able to enhance promoter induction by vincristine through a specific point mutation [T→C(+ 103)] at the site of a known drug response element within the MDR1 promoter construct. Using the mutant MDR1 promoter, expression levels were significantly higher than wild-type in the resistant cell line compared to the sensitive cell line.

The investigators then linked chemoresponsive elements of the MDR1 promoter to a human TNF-α cDNA delivered by a retroviral vector for transduction of human mammary and colon carcinoma cell lines [22]. Using this vector system, selective activation of TNF-α production was induced by administration of chemotherapy. In both the sensitive and the resistant cell lines, exposure to vincristine, taxol and doxorubicin resulted in TNF-α induction. There was also a significant increase in cytotoxicity compared with either doxorubicin or vector treatment alone. To further assess this approach, human MCF-7 breast carcinoma cells transduced with the modified MDR-TNF-α vector were transplanted in the flank of athymic mice [23]. Once tumors reached 6 mm in size, animals were treated with doxorubicin to induce TNF-α expression within the tumor. TNF induction was approximately 25-fold above that in control MCF-7 tumors 24 h after doxorubicin treatment. Serum from the same mice revealed no significant elevation in systemic TNF-α levels, consistent with local production of the cytokine. Tumor growth was similar for tumors derived from the parental cell line as compared to the transduced cells. However, following two injections of doxorubicin, a significant regression of the transduced, compared to the control tumors, was observed that lasted for over 4 weeks. This study demonstrated the potential for a chemoinducible gene therapy strategy based upon the enhanced MDR1 promoter vector. Development of the Egr-1 and MDR1 promoter strategies provided the experimental basis for initial clinical trials of chemoinducible gene therapy aimed at enhanced local control through transgene expression with the benefits of simultaneous systemic therapy.

Clinical trials with TNFerade and chemoradiotherapy

TNFerade, a second-generation E1-, partial E3- and E4-deleted adenoviral vector carrying the transgene encoding for human TNF-α downstream of the Egr-1 promoter, was first tested in combination with radiation therapy in two phase I trials for patients with various solid tumors [24].

Compared with that used in preclinical studies, TNFerade has an additional deletion (E4), which further attenuates the virus for clinical use by preventing viral recombination. In the first phase I study, TNFerade was delivered by intratumoral injection to 36 patients with various tumor types, including pancreatic cancer, lung cancer, head and neck cancer, colorectal cancer, and melanoma. Patients received weekly intratumoral TNFerade injections for 6 weeks at dose levels ranging from 4×10^7 to 4×10^{11} particle units (p.u.) followed by radiotherapy, which began during week 2 of the study. There were no dose-limiting toxicities observed, and only grade 2 constitutional symptoms such as fever, chills, and pain at the injection site were reported. Serum TNF-α levels during treatment remained below 50 pg/ml. Of the 30 evaluable patients, five patients demonstrated a complete response. Three of these patients had localized melanoma. Sixteen more patients had objective tumor responses (nine partial and seven minimal responders). Additionally, patient samples of blood, urine and sputum all tested negative for the presence of virus. A second phase I study evaluated the safety of intratumoral administration of TNFerade in combination with radiotherapy in patients with large extremity soft tissue sarcoma [14]. TNFerade was administered to 14 patients by intratumoral injection for 5 weeks with concomitant radiation (50 Gy). Tumor size at the onset of the study averaged approximately 215 cm^2 (range 25–675 cm^2). Of the 13 evaluable patients enrolled in the study, 11 were given the treatment in the neoadjuvant setting and two for palliation. Among the 11 patients who underwent resection, two patients demonstrated a complete pathologic response and eight patients a partial response. Of the patients who were treated for palliation, one patient had stable disease and another had a partial response. Pathologic evaluation of resected specimens revealed negative margins for all tumors. Diffuse intratumoral necrosis was noted in resected tumors and four of eight patients with a partial response showed more than 95% tumor necrosis on pathologic examination. These phase I trials confirmed that TNFerade and radiation are well tolerated, and suggested that TNFerade and radiotherapy may be effective for large tumors.

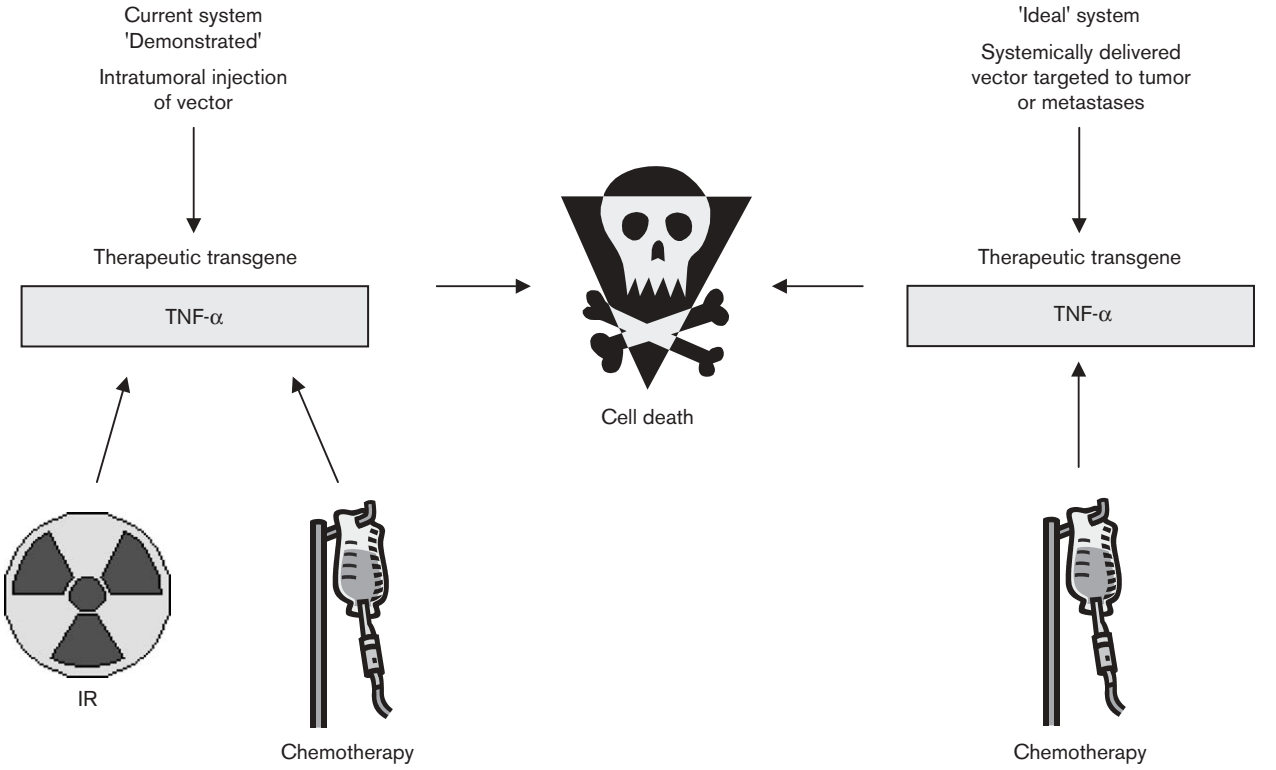
Phase I–II trials of TNFerade in combination with both radiation and chemotherapy were initiated for patients with locally advanced pancreatic or esophageal cancer. In the phase I-II trial for patients with locally advanced pancreatic cancer, intratumoral injections of TNFerade were delivered by endoscopy or percutaneously injection under ultrasound or computed tomography guidance (www.genvec.com). Doses ranged from 4×10^9 to 1×10^{12} p.u. Radiotherapy (50.4 Gy) and 5-fluorouracil (5-FU) were given for 5 days at 5 weeks after intratumoral injection of TNFerade. TNFerade was well tolerated and the maximum tolerated dose was 4×10^{11} p.u. Dose-limiting toxicities were observed at the 1×10^{12} dose (two patients experienced pancreatitis and one patient ascending cholangitis). Plasma TNF-α levels were elevated (above 50 pg/ml) in two of the evaluable 23 patients and returned to baseline levels following the completion of the therapy. At 3 months following treatment initiation, 70% of the patients had no progression and 34% of the patients demonstrated a more than 25% reduction in tumor size. TNFerade was more effective in a dose-dependent fashion. Of the seven patients who underwent resection, six had negative pathologic margins, which included four of the five patients who received the maximum tolerated dose of 4×10^{11} p.u. Also at the interim analysis, five of the 11 patients are still alive, including three patients beyond 21 months who were treated with the maximum tolerated TNFerade dose.

Patients enrolled in the phase I–II trial with locally advanced (stage 2 or 3), yet resectable, adenocarcinoma or squamous cell carcinoma of the esophagus received a 5-week course of chemoradiation (45 Gy, 5-FU and cisplatin) and intratumoral injection of TNFerade by endoscopic ultrasound or endoscopy (4×10^8 to 4×10^{10} p.u.). As found in the pancreatic cancer study, TNFerade was well tolerated by patients and at the completion of therapy there were no serum elevations of TNF-α. Following the neoadjuvant combined modality therapy, patients underwent resection and the specimens were evaluated for pathologic response. Six of seven patients receiving 4×10^8, three of four who received 4×10^9 and four of five patients who received 4×10^{10} p.u. underwent resection. The complete pathologic response rate for this cohort of patients was 38%. This study was closed early due to a higher rate of thromboembolic events in the patients treated with the highest dose (4×10^{11}pu) of TNFerade (N. N. S., personal observation).

The future for chemoinducible gene therapy

The use of adenoviral vectors for the local/targeted delivery of cytotoxic cytokines has potential benefit as a novel therapy for solid tumors in combination with DNA-damaging agents. The results obtained with TNFerade in combination with both radiotherapy and chemotherapy for the treatment of advanced local disease are promising. The establishment of chemotherapy as a transcriptional targeting agent carries with it the theoretical benefit of treating distant disease with a systemically administered agent. For example, chemotherapy in combination with an inducible vector system can be used to treat tumors like glioblastoma that recur following radiotherapy and cannot be treated with additional radiation [19]. Finally, as specific targeted adenoviruses and other delivery vectors are developed [25], a systemically administered vector carrying a cytotoxic transgene can be 'activated' by the administration of chemotherapy leading to localized

Fig. 3



Cancer treatment with gene therapy and DNA-damaging agents. The findings that radiation and chemotherapy 'activate' transgenes of locally delivered adenoviruses provide the basis for an ideal system, which would utilize a systemically administered, targeted viral vector that could be activated by subsequent administration of chemotherapy treating both primary and metastatic tumors.

tumor toxicity (of primary or metastatic lesions) without systemic effects (Fig. 3).

References

1 Advani S, Weichselbaum RR, Kufe DW. Gene therapy. In: *Cancer medicine*. Kufe D, Pollack RE, Weichselbaum RR, Bast RC, Gansler TS, Holland JF, *et al.*, editors. Hamilton, BC: Decker; 2003. pp. 981–997.

2 Weichselbaum RR, Kufe D. Gene therapy of cancer. *Lancet* 1997; **349**(**Suppl 2**):SII10–SII12.

3 Hallahan DE, Mauceri HJ, Seung LP, Dunphy EJ, Wayne JD, Hanna NN, *et al.* Spatial and temporal control of gene therapy using ionizing radiation. *Nat Med* 1995; **1**:786–791.

4 Weichselbaum RR, Hallahan DE, Sukhatme VP, Kufe DW. Gene therapy targeted by ionizing radiation. *Int J Radiat Oncol Biol Phys* 1992; **24**: 565–567.

5 Mauceri HJ, Hanna NN, Wayne JD, Hallahan DE, Hellman S, Weichselbaum RR. Tumor necrosis factor-α (TNF-α) gene therapy targeted by ionizing radiation selectively damages tumor vasculature. *Cancer Res* 1996; **56**:4311–4314.

6 Staba MJ, Mauceri HJ, Kufe DW, Hallahan DE, Weichselbaum RR. Adenoviral TNF-alpha gene therapy and radiation damage tumor vasculature in a human malignant glioma xenograft. *Gene Ther* 1998; **5**:293–300.

7 Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factor-α mRNA after cellular exposure to ionizing radiation. *Proc Natl Acad Sci USA* 1989; **86**:10104–10107.

8 Lienard D, Ewalenko P, Delmotte JJ, Renard N, Lejeune FJ. High-dose recombinant tumor necrosis factor-α in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. *J Clin Oncol* 1992; **10**:52–60.

9 Thom AK, Alexander HR, Andrich MP, Barker WC, Rosenberg SA, Fraker DL. Cytokine levels and systemic toxicity in patients undergoing isolated limb perfusion with high-dose tumor necrosis factor, interferon gamma, and melphalan. *J Clin Oncol* 1995; **13**:264–273.

10 Kufe D, Weichselbaum R. Radiation therapy: activation for gene transcription and the development of genetic radiotherapy-therapeutic strategies in oncology. *Cancer Biol Ther* 2003; **2**:326–329.

11 Datta R, Rubin E, Sukhatme V, Qureshi S, Hallahan D, Weichselbaum RR, *et al.* Ionizing radiation activates transcription of the EGR1 gene via CArG elements. *Proc Natl Acad Sci USA* 1992; **89**:10149–10153.

12 Chung TD, Mauceri HJ, Hallahan DE, Yu JJ, Chung S, Grdina WL, *et al.* Tumor necrosis factor-α-based gene therapy enhances radiation cytotoxicity in human prostate cancer. *Cancer Gene Ther* 1998; **5**:344–349.

13 Rasmussen H, Rasmussen C, Lempicki M, Durham R, Brough D, King CR, *et al.* TNFerade biologic: preclinical toxicology of a novel adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor-α gene. *Cancer Gene Ther* 2002; **9**:951–957.

14 Mundt AJ, Vijayakumar S, Nemunaitis J, Sandler A, Schwartz H, Hanna N, *et al.* A phase I trial of TNFerade biologic in patients with soft tissue sarcoma in the extremities. *Clin Cancer Res* 2004; **10**:5747–5753.

15 Sodhi A, Gupta P. Increased release of hydrogen peroxide (H_2O2) and superoxide anion (O_2^-) by murine macrophages *in vitro* after *cis*-platin treatment. *Int J Immunopharmacol* 1986; **8**:709–714.

16 Senturker S, Tschirret-Guth R, Morrow J, Levine R, Shacter E. Induction of apoptosis by chemotherapeutic drugs without generation of reactive oxygen species. *Arch Biochem Biophys* 2002; **397**:262–272.

17 Park JO, Lopez CA, Gupta VK, Brown CK, Mauceri HJ, Darga TE, *et al.* Transcriptional control of viral gene therapy by cisplatin. *J Clin Invest* 2002; **110**:403–410.

18 Lopez CA, Kimchi ET, Mauceri HJ, Park JO, Mehta N, Murphy KT, *et al.* Chemoinducible gene therapy: a strategy to enhance doxorubicin antitumor activity. *Mol Cancer Ther* 2004; **3**:1167–1175.

19 Yamini B, Yu X, Gillespie GY, Kufe DW, Weichselbaum RR. Transcriptional targeting of adenovirally delivered tumor necrosis factor-α by temozolomide in experimental glioblastoma. *Cancer Res* 2004; **64**:6381–6384.

20 Sikic BI, Fisher GA, Lum BL, Halsey J, Beketic-Oreskovic L, Chen G. Modulation and prevention of multidrug resistance by inhibitors of P-glycoprotein. *Cancer Chemother Pharmacol* 1997; **40**(**Suppl**):S13–S19.
21 Stein U, Walther W, Shoemaker RH. Vincristine induction of mutant and wild-type human multidrug-resistance promoters is cell-type-specific and dose-dependent. *J Cancer Res Clin Oncol* 1996; **122**:275–282.
22 Walther W, Stein U, Fichtner I, Naundorf H, Alexander M, Shoemaker RH, *et al*. *In vivo* evaluation of a drug-inducible vector system for the combined gene- and chemotherapy of cancer. *Adv Exp Med Biol* 1998; **451**: 139–144.
23 Walther W, Stein U, Fichtner I, Alexander M, Shoemaker RH, Schlag PM. Mdr1 promoter-driven tumor necrosis factor-α expression for a chemotherapy-controllable combined *in vivo* gene therapy and chemotherapy of tumors. *Cancer Gene Ther* 2000; **7**:893–900.
24 Senzer N, Mani S, Rosemurgy A, Nemunaitis J, Cunningham C, Guha C, *et al*. TNFerade biologic, an adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor-α gene: a phase I study in patients with solid tumors. *J Clin Oncol* 2004; **22**:592–601.
25 Everts M, Curiel DT. Transductional targeting of adenoviral cancer gene therapy. *Curr Gene Ther* 2004; **4**:337–346.