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Conditional gene targeting for cancer gene therapy

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Abstract

Current treatment of solid tumors is limited by severe adverse effects, resulting in a narrow therapeutic index. Therefore, cancer gene therapy has emerged as a targeted approach that would significantly reduce undesired side effects in normal tissues. This approach requires a clear understanding of the molecular biology of both the malignant clone and the biological vectors that serve as vehicles to target cancer cells. In this review we discuss novel approaches for conditional gene expression in cancer cells. Targeting transgene expression to malignant tissues requires the use of specific regulatory elements including promoters based on tumor biology, tissue-specific promoters and inducible regulatory elements. We also discuss the regulation of both replication and transgene expression by conditionally-replicative viruses. These approaches have the potential to restrict the expression of transgenes exclusively to tissues of interest and thereby to increase the therapeutic index of future vectors for cancer gene therapy.

Keywords: Cancer gene therapy; Gene expression; Regulatory elements

Contents

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1. Introduction

The therapeutic index of currently available modalities for most metastatic and locally advanced malignancies is low. Consequently, cancer patients often witness severe adverse effects, most notably myeloid stem cell suppression. Available cytotoxic agents have a cancer cell-to-normal cell therapeutic ratio as low as 2:1 to 6:1. Therefore, innovative strategies for the treatment of cancer are highly desired. Recently, novel strategies using gene therapy strategies have been reported to achieve tumor-selectivity from 100:1 [1] to 10,000:1 [2]. The key steps of these agents to achieve tumor targeting are based on recent advances in the molecular characterization of cancer biology. Various gene therapy approaches have been employed to address cancer, whereby direct toxicity and immune modulation by transgene expression play a major role. In this regard, adenovirus is a leading vehicle for gene delivery into tumor cells. While experimental models of gene therapy using non-replicating adenoviruses have shown some tumor-selective anti-cancer effect in animal models [3], clinical trials have failed to result in substantial tumor eradication. Therefore, efficient in vivo treatment of cancer requires the introduction of more powerful agents to induce a strong bystander effect. As patients' safety requires tumor selectivity, tight control over these agents is of paramount importance. In this regard, targeting transgene expression to cancer gene therapy is an area of intense research.

Therapeutic targeting to malignant tissues may be extrinsic or intrinsic [4]. Extrinsic targeting implies surgery, irradiation or drug delivery to tumors. Intrinsic targeting involves transductional targeting (selective uptake of vectors) or transcriptional targeting (selective expression of transgenes).

In transductional targeting the interaction between the vector and the cell surface determines the targeting efficacy. Transductional targeting may employ binding to membrane antigens [5] or protease-rich tumor cell surfaces [6], modification of viral envelope protein sequences, pseudotyped viruses or antibodies as mediators of viral infection [7–11]. Tumor vasculature may also be a transductional target [12]. Beyond transductional targeting, construction of viral vectors should apply conditional and cell-specific regulatory elements in order to control transgene expression (transcriptional targeting). In this scenario, transcription of the gene of interest occurs only under specific intracellular conditions where a specific promoter is activated. These regulatory signals may be either constitutive or inducible, native or foreign, but are required to maintain tumor-tissue specific activity rather than become leaky and induce transgene expression in normal tissues [13]. As other techniques of blocking gene transcription such as gene disruption are inefficient [14], transcriptional regulation of transgenes in cancer gene therapy is thus essential.

Transcriptional regulation is the primary event in the process of DNA transcription into messenger RNA and the following translation into a protein. The interaction between enhancer and promoter elements embedded within the DNA and protein transcription factors results in regulation of transcription. Some promoters are more tissue-specific (prostatic-specific antigen (PSA) for the prostate and tyrosinase for melanocytes) and some are less (carcinoembryonic antigen (CEA)). A promoter is usually considered an appropriate candidate for cancer gene therapy if its activity is upregulated in the tumor tissue or if the target tissue is dispensable or replaceable (prostate, breast, melanocytes, endocrine). The essential criteria for promoter selection include the strength of the promoter, tissue specificity and its size.

Viral-based sequences have a strong promoter activity but are often attenuated in vivo, in addition to their lack of specificity. Therefore, appropriate promoter candidates include regulatory elements that are already expressed by the malignant cell, tissue-specific promoters or externally-inducible sequences [15,16]. Unfortunately however, these candidate promoters often lack either sufficient activity or specificity, or both. To address promoter potency, promoters and enhancers that retain cell-specific function may be linked to transactivators. For example, a weak tissue-specific promoter may be used to drive expression of the GAL4/VP16 fusion protein, which in turn transactivates a minimal synthetic promoter, GAL4/TATA upstream of the transgene. This construct can amplify transgene expression in a relatively tissue-specific manner [17]. Additional strategies to enhance promoter activity in malignant

tissues include the use of cell-cycle elements, normal or abnormal tissue differentiation factors, hormones, cytokines, chemicals or physical stimuli.

A convenient classification of candidate promoters for cancer gene therapy involves tumor-associated promoters, tissue-specific promoters, and inducible promoters. These classes will be further discussed, as well as the role of transcriptional regulation of replication-competent viruses that have recently become a significant arm of cancer gene therapy.

2. Promoters based on tumor biology (Table 1)

The highly-complex biological nature of tumors, may hinder tumor-selectivity of candidate promoters. However, it is nevertheless tempting and challenging to address key events in tumorigenesis to target gene therapy vectors to various types of tumors. In this regard, one of the essential components of cellular immortality and cancer is telomerase.

High expression of this enzyme is found in malignant tumors but not in normal tissues, except for stem cells and germ cells. RNA-dependent DNA polymerase, synthesizing new telomeric repeats at the end of chromosomes, is a very common feature in human cancers. Telomerase is thought to be essential for the maintenance of the proliferative capacity of tumor cells, thereby representing an attractive target for new anti-cancer therapies. The human telomerase reverse transcriptase is regulated primarily at the transcriptional level [18]. The human telomerase reverse transcriptase (hTERT) promoter is apparently a strong and tumor-selective promoter with a promising potential for targeted cancer gene therapy. It efficiently induces reporter and apoptotic genes expression in tumor cells but not in normal tissues tested, excluding stem and germ cells [19]. Additionally, expression of diphtheria toxin A-chain (DT-A) gene was induced by the telomerase promoter in telomerase-positive urinary bladder and hepatocellular carcinoma cancer cell lines. However, in a fibroblast telomerase-negative cell line transgene expression was also induced, albeit without apparent toxic effects. The telomerase promoter therefore merits more studies in regard to its specificity to malignant tissues [20].

Tumor vasculature is an important target for anti-cancer gene therapy. Its advantages include good accessibility to systemically delivered therapy and comparative homogeneity across solid tumor types [21]. High levels of the vascular endothelial growth factor (VEGF) are correlated with worse prognosis, at least for breast cancer patients. VEGF activity is mediated by two high-affinity receptors. These ligand-stimulated tyrosine kinases, VEGFR-1/flt-1 and VEGFR-2/flk-1, are induced in a tumor stage-dependent manner during cancer progression and are exclusively expressed in tumor vascular endothelial cells. These observations suggest that VEGF-receptors are promising targets for tumor endothelial cell specific therapy [22].

Consequently, transcriptional targeting in endothelial cells as part of an anti-vasculature approach to cancer treatment may exploit the strong promoter activity of the 939-bp Flk-1 promoter fragment and of an enhancer element located in a 2.3-kb fragment upstream. This promoter was used to induce a tumor

Table 1
Transcriptional regulation of cancer gene therapy based on aberrant tumor biology

Transcriptional mechanism	Promoter	Target tumor	Transgene	Reference
Aberrant tumor biology	Telomerase	Urinary bladder and hepatocellular carcinoma	Diphtheria toxin	[20]
	FLK-1	Melanoma, fibrosarcoma and breast tumor vessels	Reporter gene	[22,23]
	E-Selectin	Tumor vasculature	TNF-α	[21]
	VEGF	Lung carcinoma	HSV-TK	[45]
	Hexokinase II	Lung carcinoma	HSV-TK	[51]
	c-erbB2	Breast and pancreas cancers	CD	[52]
	c-Myc	Small cell lung cancer	CD	[53]
	L-Plastin	Ovarian carcinoma	Reporter gene	[55]
	SLPI	Lung and ovary cancers	HSV-TK	[59]

endothelium-specific reporter gene expression in transgenic mice, independently of the tumor type [23]. Other studies also indicate that targeting the VEGF receptor/ligand system is a rational approach to inhibit tumor growth and prolong survival [24]. The human prepro-endothelin-1 promoter could also show specificity for breast microvascular endothelial cells when used to drive beta-galactosidase expression by a recombinant retroviral vector [25].

Cell adhesion, playing an important role in tumor vasculature and cancer metastasis, is partially mediated by E-selectin and its carbohydrate ligands, Le(a) and Le(x). E-selectin expression is very low in normal adult blood vessels, but is significantly elevated in newly formed tumor capillaries. E-selectin promoter was shown to confer transcriptional specificity of a reporter gene expression in tumor vasculature [26] and to drive endothelial-specific murine TNF-alpha expression by a retrovirus [21]. Interestingly, as E-selectin is upregulated by TNF-α, it may launch a self-propagating process.

The switch from a local tumor to a metastatic cancer signals the worst prognosis for cancer patients. Relatively few genes have been implicated in this switch. Recent data indicates that heparanase [27], as well as RhoC, fibronectin and thymosin β4 [28], may be associated with the metastatic potential of tumors. Heparan sulfate cleavage by endoglycosidic heparanase affects a variety of biological processes, leading to a metastatic phenotype.

RhoC, fibronectin and thymosin β4 are involved in extracellular matrix assembly and regulation of the actin-based cytoskeleton. In this regard, promoters/enhancers of these invasion-related genes may offer transcriptional targeting of metastatic cancers. Endoglin (CD105), a glycoprotein member of the transforming growth factor beta (TGF-beta) receptor family expressed predominantly on proliferating human endothelial cells, is also correlated with metastatic potential, at least in breast cancer [29,30], and with local invasion [31]. Endoglin, also expressed on normal and neoplastic cells of the melanocytic lineage, is found in a wide array of childhood brain tumors [32] and is upregulated in tumor angiogenesis [33]. Further characterization of endoglin activation may thus enable transcriptional vascular targeting. The mechanism of endoglin association with vascular proliferation may relate to an arginine–glycine–aspartate (RGD) sequence, a feature generally associated with extracellular matrix proteins that interact with cell-membrane integrins. Endoglin might be implicated in tumor angiogenesis either directly, by binding to integrins in cell–cell stromal adhesion events, or indirectly by regulating the level of adhesion between certain integrins and their receptors. As various integrins have a major role in cellular invasion and metastasis [34], specific integrins could be good candidates for tumor targeting. Tumor invasion and metastasis depend to a large extent on the interaction of integrins and members of the protease-activated receptor family with various RGD-containing ligands [35], including fibronectin [36] and thrombin [37]. Among the integrin family, alpha-V beta-3 integrin is probably involved in tumor metastasis [38] and may be triggered by RGD to activate a cascade of essential signaling cues to the cells, resulting in their physical linking to the actin filaments [39] and phosphorylation of proteins like focal adhesion kinase (FAK) and paxillin [40].

A growing number of studies indicate that signals driven by integrins act in concert with signals initiated by the G-protein-coupled receptors and with receptors for tyrosine kinase to promote the pathological tumor cell invasion process, on the one hand, and physiological activities like angiogenesis and wound healing on the other [41]. Considering the potent anti-cancer effect of inhibiting tyrosine kinase [42], tumor transcriptional targeting may also benefit from focusing on the molecular events resulting in local invasion and metastases [43]. In this regard, distinction between physiological and malignant phenotypes is a critical step whereby transcriptional or post-transcriptional targeting may specifically limit the invasive potential of tumors.

Many genes controlling tumor biology are oxygen regulated, and new ones are constantly added to the growing list of hypoxia-induced genes. Of specific importance are hypoxia-responsive transcription factors, as they can modulate the expression of numerous different genes. Similarly, growth factors which govern the formation of new blood vessels or control blood flow are vitally important for both the maintenance of the primary tumor and its metastases. Since hypoxic cells have a reduced transcription rate, promoter upregulation by hypoxia may result in enhanced efficacy. Hypoxia-inducible factor-1 (HIF-

1) has been shown to mediate the transcriptional activation of its target genes in response to oxygen concentration, most likely via a pathway involving a specific oxygen sensor. While HIF-1 activity is regulated by proteolysis, rather than at the level of transcription or translation, HIF-1 plays a critical role in the response of diverse target genes involved in cellular growth and metabolism. Thus, HIF-1 is a prime candidate regulator molecule for the role of coordinating vascular oxygen supply with cellular growth and energy metabolism [44]. HIF is a key transcriptional regulator of the cellular response to hypoxia that is upregulated in many common cancers.

There is an extensive array of HIF target genes, whose regulatory elements may provide a new opportunity for transcriptional targeting to hypoxic tumors. In this regard, the promoter region encompassing the hypoxia response element (HRE) of the mouse VEGF gene has been used to drive the HSV-TK gene expression under hypoxic conditions and to efficiently kill highly-metastatic lung carcinoma cells [45].

Candidate tumors for transcriptional targeting of HIF-1-responsive elements include breast and colon cancers [46], and possibly also clear-cell renal carcinoma, which commonly lacks the von Hippel–Lindau tumor suppressor activity that targets HIF for oxygen dependent proteolysis.

A prominent hallmark of cancer biology is unregulated cellular proliferation. The retinoblastoma (Rb) family of proteins and their upstream regulators, namely cyclin D, cyclin D kinase 4, and $p16^{INK4a}$, regulate the cellular G1 checkpoint. P21, functioning upstream of RB, is upregulated by the tumor suppressor protein p53, and is important for maintaining cell cycle arrest. It exerts its effect by inhibiting cyclin D kinases that promote cell progression into S phase by phosphorylating and inactivating Rb [47]. Hypophosphorylated Rb binds to and inactivates the cellular growth factor E2F that plays a pivotal role in the coordinated transactivation of cell cycle-regulatory genes, leading to cell proliferation [48]. Therefore, E2F-responsive transgene expression is expected to be relatively tumor-selective. In addition to *c-myc* and cdc2, E2F-1 promoter is also E2F-responsive and results in apoptosis. Tumor suppression by pRB has been linked to its ability to repress E2F-responsive promoters such as the E2F-1 promoter. Thus, E2F-responsive promoters should be more active in tumor cells relative to normal cells because of an excess of free E2F and loss of pRB/E2F repressor complexes.

As de-repression of E2F-1 promoter occurs in cancer tissues, it was exploited to design viral vectors mediating relatively tumor-selective gene expression in gliomas. An adenoviral vector where a toxic gene was driven by the E2F-1 promoter could eradicate established gliomas with a good therapeutic ratio [49].

Impaired glucose metabolism is another feature of cancer. The glucose transporter, GLUT3, is induced in vascular, rapidly-growing tumors unlike slow-growing tumors [50]. Hexokinase type II (HK II), the enzyme catalyzing the first committed step of glycolysis is overexpressed in tumors, where it is no longer responsive to normal physiological inhibitors, e.g., glucagon. This feature was used to selectively express a reporter gene under the control of HK II in lung cancer cell lines and to induce selective toxicity following infection with a non-replicating adenoviral vector expressing the herpes simplex thymidine kinase (*tk*) under the control of HKII [51].

Activation of oncogenes results in malignant transformation. Therefore, molecular targeting may exploit their activation to induce an anti-cancer effect. The *c-erb*B2 oncogene is involved in some types of breast, gastric and pancreatic carcinomas. Its promoter is activated in these tumors by the transactivator OB2-1. This feature was exploited to construct the *c-erb*B2 promoter with a cytosine deaminase (*cd*) gene to selectively kill breast and pancreatic cell lines overexpressing *c-erb*B2 [52]. A similar approach using *c-myc* was applied for small cell lung carcinoma in vitro [53].

The promoter of L-plastin, a human actin-binding protein, has recently been identified as a promising candidate for targeting gene expression to several carcinomas. L-plastin is constitutively and abundantly expressed in malignant epithelial cells but is not expressed in normal tissues, except for low-level expression in mature hematopoietic cells.

Because adenoviral vectors infect early hematopoietic multilineage precursor cells only poorly if at all, transcription of therapeutic genes in cells infected by an adenoviral vector is expected to be tumor

specific. L-plastin-positive ovarian carcinoma would thus be a good target for such a vector, designed to untarget the normal mesothelial cells of the peritoneal cavity [54,55].

Importantly, the relative activity of L-plastin in malignant breast and ovarian cancers and in fibrosarcomas is high in regard to normal fibroblasts. Additionally, it has steroid hormonal binding sites, rendering it subject to pharmacological modulation.

A common feature of head and neck, breast, colon, ovarian, endometrial, bladder and lung cancers, in particular adenocarcinoma, is the expression of the secretory leukoproteinase inhibitor (SLPI) [56]. However, SLPI mRNA is normally also found in the islets of Langerhans [57] and in normal bronchial epithelium [58]. A 1358 bp fragment of the SLPI promoter was used to drive *tk* gene in a plasmid context. Transfection of this construct into SLPI-positive and negative lung and ovarian cancer cell lines selectively killed the SLPI-positive cell lines following ganciclovir administration [59]. In this regard, further selectivity may be achieved by the interferon regulatory factor (IRF)-1. IRF-1 is involved in cell growth regulation and represses SLPI expression following binding to responsive sites within its promoter [60], therefore harboring the potential to inhibit SLPI-driven transgene in normal and inflammatory tissues.

The anti-apoptotic gene bcl-2 has recently been found to be transcriptionally activated in non-small cell lung carcinoma [61], breast cancer cells [62] and some Wilms' tumors [63]. These findings complement the pathophysiological role of bcl-2 in chronic myeloid leukemia, whereby it serves as a downstream target gene for the Ras signaling pathway in transformed cells [64].

Since an intact p53 represses the bcl-2 promoter [65], p53 mutation may play a role in the up-regulation of bcl-2 expression in some malignancies and may untarget its expression in normal tissues.

The quest for a comprehensive transcriptional targeting approach for cancer gene therapy will probably not yield a single tumor-associated gene regulatory element. Nonetheless, as some genes, such as COX-2, epidermal growth factor (EGFR) and *c-erb*B2 (part of the EGFR family) are linked in a positive feedback cycle in neoplastic proliferation [66], predicating upon certain common features of malignancy may result in a more broad-spectrum conditional gene targeting. In this regard, any discussion of tumor biology-based approaches for cancer gene therapy would not be complete without a short description of post-transcriptional targeting using antisense oligodeoxynucleotides (ODN). ODN harbor a potential for a highly specific gene targeting, but their delivery is nevertheless a rate-limiting factor. ODN are short sequences of complementary DNA synthesized exactly to complement specific mRNA. As RNA-DNA hybridize message translation is interrupted. The potential advantage of anti-oncogene therapy rests in selectivity for mutant cells while sparing normal cells. Targeting *bcr/abl* transcripts after the juxtaposition by translocation has occurred, suppresses Philadelphia leukemic cell proliferation in chronic myelogenous leukemia (CML) cell lines [67] and prolong the survival of SCID mice [68]. Two distinct ODN, targeting both *bcr/abl* and *c-Myc,* a second oncogene involved in signal transmission in CML, are even more potent in reducing the leukemic cell load [69]. *c-Myb* antisense ODN targeting has also been shown to inhibit growth of various leukemic cell lines, depending on this gene for proliferation [70].

Clinical experience with *c-Myb* and *bcr/abl* antisense ODN for CML phase I trials showed that this therapeutic modality is safe [71], while *bcl-2* targeted ODN led to a slight improvement in a limited number of patients with non-Hodgkin's lymphoma [72].

Recently, human non-Hodgkin's lymphoma was eradicated in SCID mice by *bcl-2* antisense ODN combined with low-dose cyclophosphamide [73]. Antisense ODN have also been produced to target synthesis of tumor-endogenous immunogenic-masking proteins, such as IGF-1 and TGF-β [74,75].

3. Tissue-specific promoters (Table 2)

The utility of tissue-specific promoters is limited by their natural activity in normal tissues. However, as some tissues are dispensable, promoters that are active in these tissues may be exploited to selectively activate therapeutic transgenes. Optimal candidate tissues include melanocytes, prostate, breast, endocrine and exocrine tissues. However, malignancies in

Table 2
Transcriptional regulation of cancer gene therapy based on tissue-specificity

Transcriptional mechanism	Promoter	Target tumor	Transgene	Reference
Tissue-specificity	PSA, Kallikrein	Prostate	Reporter gene or nitroreductase	[79]
	Tyrosinase	Melanoma	Reporter gene	[81]
	Tyrosinase	Melanoma	IL-2	[83]
	CEA	HCC	CD	[88]
	αFP	HCC	HSV-TK	[3]
	c-erbB2	Pancreas	CD	[91,92]
	Amylase	Pancreas	Reporter gene	[96]
	SP-B	Lung	Adenoviral E4	[99]
	CEA	Pancreas	Dominant negative ***ras*** mutant	[95]
	Grp	Small cell lung carcinoma	HSV-TK	[100]
	AVP	Small cell lung cancer	Reporter gene	[103,104]
	Immunoglobulin heavy chain	B lymphomas	Diphtheria toxin	[105]
	AP-2	Breast cancer	CD	[112]
	α-lactalbumin	Breast cancer	CD	[113]
	Osteocalcin	Osteosarcoma	HSV-TK	[118]
	Prolactin	Prolactinoma	HSV-TK	[121]

other tissues often involve degeneration and activation of embryonal or other abnormal genes, therefore enabling a range of transcriptional targeting of cancer involving essential tissues as well.

3.1. Prostate carcinoma

Prostate-specific antigen (PSA) is expressed at a high level in the luminal epithelial cells of the prostate and is absent or expressed at very low levels in other tissues. The complete PSA promoter is 5837 bp long and although usually regulated by androgens [76], it may retain its activity in an androgen-free environment [77] and also positively respond to IGF-1 [78]. However, the minimal 642 bp PSA promoter is weak in both PSA-positive or PSA-negative cells and does not respond to androgenic stimuli.

The human kallikrein-2 (hKLK2) is also expressed predominately in the prostate, and is transcriptionally up-regulated by androgens. Moreover, it shares 78% homology with the PSA promoter [2]. Placing a 1455-bp PSA enhancer sequence upstream of either the PSA or hKLK2 promoters increases transgene expression 20-fold in PSA-positive cell lines but not in the PSA-negative lines. Tandem duplication of the PSA enhancer increases expression approximately 50-fold while retaining tissue-specific control [79]. A higher efficiency can be achieved by coupling the PSA promoter to a yeast promoter [80].

3.2. Melanoma

Specificity for malignant melanoma may be conferred by the human tyrosinase promoter. In vitro and in vivo melanoma transduction by constructs driven by the tyrosinase promoter results in tumor-selective transgene induction and complete tumor regression [81,82]. Similarly, a construct consisting of 209 bp of the human tyrosinase promoter linked to two enhancer elements was demonstrated to drive high-level, melanoma-specific expression of a reporter gene in transient transfection assays. The murine tyrosinase promoter-enhancer expression cassette expressed by an adenoviral vector maintains transcriptional specificity for pigment cell lineages, especially human melanoma cell lines [83]. Melanomas often express abundantly metalloproteinases. Adenoviral-mediated gene delivery of tissue inhibitors of metalloproteinase significantly reduces local invasion in vitro [84], possibly by apoptosis induction. The induction of apoptosis inhibits growth of melanoma in vivo and in vitro following heterologous expression of a dominant-negative survivin gene, under the control of the tetracycline (tet)-resistance operon.

The tet-controlled transactivator sequence downstream of the tet operon prevents transactivator binding to the tet operon and transcription of the transgene, while in the absence of tet, the transactivator upregulates its own transcription and the transgene is expressed [85].

An interesting transcriptional targeting approach to melanoma utilized replacing the Moloney Murine Leukaemia Virus (Mo-MLV) retroviral vector enhancer in the 3′-long terminal repeat (LTR) with two different lengths (2.5 kbp or 769 bp) of the murine tyrosinase promoter/enhancer. The hybrid tyrosinase-LTR was used to selectively express IL-2 in melanoma cell lines [86].

3.3. Hepatocellular carcinoma

Hepatocytes may selectively express transgenes linked to the promoters of the gluconeogenesis enzyme PEPCK and the α1-antitrypsin protease [87]. A segment of the PEPCK promoter is active in hepatocellular carcinoma and harbors insulin-, glucocorticoid- and cAMP-responsive elements. In vitro transcriptional targeting of hepatocellular carcinoma (HCC) was accomplished with α-fetoprotein (αFP) and carcinoembryonic antigen (CEA) promoters [3,88]. CEA is a common tumor marker, expressed in colon, hepatic, pancreatic and lung carcinomas. However, it may also be expressed in benign inflammatory conditions, albeit less abundantly. CEA sequence upstream of the *cd* gene can selectively sensitize HCC cells to 5 FU cytotoxicity [88]. Efficient cell killing using CEA promoter gene for CEA-producing adenocarcinoma of the lung was also reported [89]. Multimerization of sequences between −89 and −40 resulted in copy number-related increases in both expression level and selectivity for CEA-positive cells. Two enhancer regions of CEA, −13.6 to −10.7 kb or −6.1 to −4.0 kb, were identified and support high-level and selective reporter expression in CEA-positive cell lines [90].

α-fetoprotein is another relatively specific gene expressed by hepatocellular carcinoma. Its promoter has also been successfully linked to *tk* and cloned into a retroviral vector to infect and selectively kill hepatoma cells [3].

3.4. Pancreatic cancer

Pancreatic cancer can involve overexpression of *myc* or *c-erb*B2, mutational inactivation of functional p53, or activation of K-Ras. K-ras proteins are small (21-kd) proteins that normally serve as guanosine triphosphate (GTP)-regulated switches to control a diverse array of cellular signals that modulate highly regulated programs of proliferation, differentiation, and death.

Transcriptional up-regulation of the *c-erb*B2 oncogene occurs in approximately one-third of pancreatic tumors. A recombinant retrovirus incorporating a construct of a chimeric minigene consisting of the proximal *c-erb*B2 promoter linked to the gene encoding *cd*, selectively induces cell death in pancreatic cancer cell lines expressing *c-erb*B2 [91,92]. A similar approach utilized the CEA promoter, coupled to *tk* to transduce CEA-expressing pancreatic carcinoma cell lines by a retroviral vector [93] or by an adenoviral vector [94]. The CEA promoter was also used to selectively express in CEA-positive pancreatic cancer cell lines and hepatic micrometastases a dominant negative *ras* mutant, inhibiting tumor growth via induction of apoptosis [95].

The murine pancreatic amylase promoter was exploited to drive pancreatic-selective reporter gene expression in mice. Importantly, the amylase promoter retains its inducibility by dexamethasone and insulin [96].

3.5. Lung carcinoma

Lung-specific promoters have been described for the human surfactant (SP) A and B proteins. Reporter gene expression and tumor cell killing was documented for non-small cell lung cancer cell lines expressing SP-A, in contrast to non-SP-A expressing lung cancer cell lines [97]. SP-B promoter is activated in the adult to type II alveolar epithelial cells and bronchial epithelial cells, and may be a major candidate for lung cancer targeting [98].

When the SP-B promoter was used to drive E4 expression of a replicating adenovirus, it restricted viral replication and cell killing to a lung cancer cell line where the SPB promoter is active, and inhibited

the vector's oncolytic effect on liver cancer cells [99].

The gastrin-releasing peptide (GRP) is expressed in most types of small cell lung carcinoma (SCLC). An adenoviral construct containing the *Herpes simplex tk* suicide gene driven by the GRP promoter was able to sensitize GRP-expressing SCLC cells to ganciclovir in vitro and to induce complete subcutaneous SCLC tumor regression after intraperitoneal administration of ganciclovir [100].

The cyclooxygenase-2 (COX-2) promoter is activated in the lung cancer cell line A549 cells [101], as well as in many gastrointestinal cancers, and is relatively inactive in the liver [102]. An important feature of the COX-2 promoter in the lung cell lines is inducibility by IL-1-β and inhibition by dexamethasone [101], potentially rendering downstream toxic transgenes expression subject to external manipulation.

Vasopressin (AVP) is a neuropeptide physiologically synthesized in the hypothalamus but pathologically expressed by two-thirds of SCLC. Reporter gene analysis for SCLC-specific targeting revealed a minimal 65 bp fragment AVP promoter with SCLC-selective weak in vitro activity and a 199 bp AVP promoter fragment with a five-fold stronger activity, yet retaining SCLC selectivity [103,104].

3.6. Hematopoietic system

B lymphocytes, transfected with constructs of promoter and enhancer of immunoglobulin heavy chain and the diphtheria toxin gene show selective toxic expression [105].

Cytotoxic effect on T-lymphoma cells and human cervical carcinoma cell line may be achieved by engineering an adenovirus vector harboring the HSV-*tk* gene regulated by HIV LTR, used as a promoter. The gene was expressed and caused efficient cell killing after exposure to gancyclovir [106]. Interestingly, this strategy was also used to target suicide gene expression to HIV-infected lymphocytes [107]. Inverse targeting may prove ideally suited to address bone marrow suppression by chemotherapy. Retroviral vectors may allow selective transduction of receptor-negative cells in a mixed cell population. Inverse targeting strategy is useful for mixed populations of carcinoma and hematopoietic cells to achieve selective transduction of transduction of hematopoietic cells with *MDR-1* gene (encoding the efflux protein p170), while exposing carcinoma cells to chemotherapy [108]. In this regard, targeting expression of retroviral transgenes to specific progenies of transduced hematopoietic stem cells may use enhancer replacement whereby the constitutive viral enhancer in the U3 region of the 3′ LTR is replaced by an autoregulatory enhancer of the erythroid-specific GATA-1 transcription factor gene [109].

3.7. Breast carcinoma

The mammary tissue is unique in expressing β-casein and acidic whey protein genes, harboring repressor elements within their promoters, thereby downregulated in non-mammary tissues [7]. The expression of milk proteins is regulated by complex promoters, comprising both positive and negative effect on gene expression, under hormonal regulation [110]. Since breast carcinoma is often a long-lasting disease with metastatic spread, identification of tissue-specific promoters is of paramount importance in order to deliver oncolytic genes systemically. Gene therapy for breast carcinoma may also be approached by tailoring a virus with affinity to this tissue, such as the mouse mammary tumor virus (MMTV).

The glucocorticoid-responsive long terminal repeats (LTR) of this retrovirus were used as promoters for dexamethasone-inducible oncolytic cytokine expression [111]. However, clinical trials were conducted for patients with recurrent breast carcinoma expressing *HER2* gene [112]. These patients were transfected with a plasmid containing *cd* gene driven by the AP-2 promoter.

Efficiency of cancer cell killing following prodrug activation is proportional to cellular *HER2* expression. The promoter of L-plastin (an actin binding protein) is constitutively highly expressed in malignant breast cancer and is not expressed in normal tissues, other than hematopoietic cells. Reporter gene activity under this promoter is relatively restricted to these malignant tissues. Moreover, L-plastin promoter may have a steroid hormone-bind-

ing site, thereby potentially conferring pharmacological modulation [55]. Transcriptional control of either the human α-lactalbumin (ALA) or ovine beta-lactoglobulin (BLG) promoter can also be used to drive *cd* expression in a breast cancer cell-specific manner resulting to efficiently eliminate subcutaneous tumor masses [113].

3.8. Brain tumors

Gene therapy of the brain is hindered by the presence of the blood–brain barrier (BBB), mandating invasive routes of administration. One way to address BBB is the transductional targeting of plasmid DNA via conjugation to monoclonal antibodies to BBB transferrin-receptor [114]. While transgene expression is feasible throughout the central nervous system, specific transcriptional regulation remains to be improved. In this regard, the neuroectodermal-specific promoters include calcineurin A-α and synapsin-I [7]. Potential use of these promoters may restrict gene expression to brain tumors that have so far been primarily addressed by immunotherapy-based gene therapy.

Candidate transgenes that are potentially subject to transcriptional regulation in the brain include β-interferon [115] and macrophage colony-stimulating factor [116]. The astrocyte-specific, glial fibrillary acidic protein (GFAP) was shown to restrict transgene expression to glial cells in cell lines, primary cultures, and CNS in vivo [117].

3.9. Bone tumors

Osteocalcin (OC), a non-collagenous bone matrix protein, is expressed in high levels by osteoblasts. The OC promoter mediates osteoblast-specific gene expression and can drive osteoblast-specific *tk* expression to eradicate murine osteosarcoma in vivo following co-administration of gancyclovir [118]. Further, this construct was tested in a model of osteosarcoma lung metastases. While a reporter gene controlled by the non-specific RSV promoter was expressed diffusely in normal lung parenchyma, the OC-adenovirus construct resulted in specific expression in OC-positive lung metastases [119].

Targeting to the skeleton for control of metastatic disease has also been proposed to exploit the OC promoter in the context of stem cell therapy [120].

3.10. Endocrine tumors

To target the lactotrophic cells degenerating to form prolactinomas, the human prolactin (PRL) promoter was encoded within a recombinant adenovirus vector to drive transgene expression of a reporter gene or *tk* in vitro and in vivo. While selective apoptosis was induced in lactotrophic cells in the presence of ganciclovir, in vivo transgene expression in the anterior pituitary gland was restricted to the reporter gene and tumor progression was not affected by the toxic gene [121].

4. Inducible promoters (Table 3)

A general problem with most of the tissue- and tumor-specific promoters is their relative weakness, thereby underscoring the need for efficient transgene activation. Therefore, inducible promoters have gained attention as mediators of transient transgene activation. During evolution various stress-genes evolved and their promoters are now considered for gene therapy transcriptional regulation. Heat, hypoxia, glucose deprivation, irradiation and chemotherapeutic agents up-regulate various genes involved in

Table 3
Transcriptional regulation of cancer gene therapy based on inducible promoters

Transcriptional mechanism	Promoter	Target tumor	Transgene	Reference
Induction	EGR-1	Glioma	TNF-α or ***TK***	[122,123]
	Hsp70	Prostate	CD + HSV-TK	[127]
	Hsp70	Breast	HSV-TK	[128]
	Hsp70	Melanoma	IL-12 or TNF-α	[129]
	Grp78	Fibrosarcoma	HSV-TK	[131]
	MDR-1	Breast	TNF-α	[132]

stress responses. Promoters of these genes are attractive for cancer gene therapy because they depend to a large extent on the biology of the tumor or are already induced by various therapeutic modalities. The stress genes upregulated in these conditions include multidrug resistance gene-1 (MDR-1), human heat-shock protein (HSP), vascular-endothelial growth factor (VEGF), irradiation-inducible *Egr-1* (early growth response gene), and the tissue plasminogen activator (*tpa*) promoters.

Irradiation-responsive promoter sequences were identified for the *tpa* and *Egr-1* genes. The first irradiation inducible promoter system used in combination with gene therapy involved the *Egr-1* promoter driving either the radiosensitizing cytokine TNF-α or *tk*. Combined therapy was more efficient than irradiation alone in vitro [122] and in vivo [123]. The heat-shock (stress) protein family is induced by a variety of environmental pressures, namely heat, irradiation, photobeam irradiation, hypoxia, acidosis, hypoglycemia and osmotic changes [124]. These conditions may exist in poorly vascularized tumors and may trigger expression of anti-cancer genes linked to the HSP70 promoter. Importantly, in p53-deficient tumor cells, HSP70 expression is up-regulated, thereby providing another means for transcriptional targeting [125,126].

A recombinant adenovirus containing the *cd-tk* fusion gene under the control of a human inducible HSP70 promoter sequence has a strong fusion gene expression after heat-shock and efficiently kills prostate cells in the presence of both 5-FC and ganciclovir [127]. The HSP70 promoter has also been employed to efficiently drive *tk* expression in vivo and inhibit growth of breast cancer subcutaneous and intraperitoneal models following hyperthermia therapy [128], to selectively induce interleukin-12 or TNF-α in melanoma tumor model by hyperthermia [129] and to drive p53 expression by photodynamic therapy [130]. The *GRP78* gene is another member of the HSP family and its promoter was used to efficiently activate reporter genes [124], and eradicate fibrosarcoma tumor masses following its incorporation into a retroviral system to drive the expression of *tk* under glucose starvation conditions [131].

The multi-drug resistance gene (*MDR-1*) encodes a membrane effluxing glycoprotein, whose expression is induced by vincristine, actinomycin D and doxorubicin. Its promoter is indirectly transactivated by these compounds and induces transcription and expression of therapeutic genes, such as TNF-α in tumors exposed to chemotherapy [132].

Chemotherapy also induces another mechanism for drug-resistance, namely activation of the glutathione detoxification system and apoptosis-controlling gene alterations (especially p53 and *bcl*-2). As the MDR-1 promoter contains heat-responsive elements, it is also activated by heat-shock [133].

A number of drug-related gene expression systems are available to control target gene transcription through the use of small-molecule inducing compounds. While the utility of such systems has been demonstrated in vitro and in transgenic mice, recent improvements are likely to make these systems more amenable for use in a therapeutic context, such as gene therapy. Rapamycin is a small molecule used in dimerization-based strategies of gene regulation [134].

Upon drug administration, dimerization occurs between a DNA-binding domain and an activation domain, resulting in transgene transcription. Another inducer, dexamethasone, a synthetic glucocorticoid, can selectively activate the p21 promoter in rat hepatoma cells via a glucocorticoid-responsive region between nucleotides -1481 and -1184. This region does not contain a canonical glucocorticoid response element but rather can confer specific dexamethasone responsiveness to heterologous promoters [135].

The Tet-controlled transcription system is comprised of Tet-off and Tet-on transcriptional regulation, derived from the *E. coli* Tet-resistance operon. The transactivator (tTA) consists of a fusion of wild-type (wt) Tet-repressor and the *Herpes simplex* VP16 activator domain. In the Tet-off state, tTA binds the Tet repressor element (TRE) and activates transcription in the absence of tetracyclin or doxycyclin. The Tet-on option was available following substitution of four amino acids at the Tet-repressor, thereby altering its binding characteristics and creating reverse repressor (rTetR).

This protein binds TRE in the presence of doxycyclin and activates transcription. Simultaneous expression of two distinct genes is possible under the control of a single TRE.

The Tet-R system was used to suppress and induce cytotoxic genes [136] and reporter genes expression [126]. The latter can select gene expression to p53-deficient tumor cells. Similarly to the R-tet, the orally bioavailable antiprogestin mifepristone can switch on gene expression in allosteric systems whereby a chimeric transactivator activates a target gene. This system was suggested to circumvent constitutive expression of various transgenes in normal tissues by drug-specific and temporal regulation of the target gene, in vitro and in vivo. Additionally, replacing the activation domain of the chimeric transactivator with a transcriptional repression domain results in inducible repression of the transgene expression [137].

Maximal level of transgene expression after induction is equal to or higher than that displayed by a plasmid driven by the CMV enhancer/promoter [138]. This system was used to express the human growth hormone in a liver-specific manner following coupling of the transactivator to the liver-specific transthyretin (TTR) promoter [139].

5. Regulatory elements for conditionally-replicative viruses (RCV)

Toxic gene or tumor suppressor gene expression from non-replicative vectors is inadequate to eradicate solid tumors in humans [140]. Consequently, replication-competent viruses (RCV) have emerged as promising therapeutic agents for cancer. The most commonly used agents in this context include adenoviruses, although retrovirus, Herpes virus [149], Reovirus [141] and Vesicular-stomatitis virus [142] have also been suggested for various malignancies. In this regard, preferential replication of Reovirus in tumor cells with activated Ras pathway is an appealing therapeutic potential deriving from a biological pathway.

The criteria governing the utility of RCV include infection efficacy, replication selectivity, lateral viral dispersion and evasion of the host immune reaction. While augmented gene transfer efficiency has been shown for advanced-generation adenoviral vectors based upon the Coxsackie adenovirus receptor (CAR)-independent cellular entry pathways [143], propagation of the virus within the three-dimensional tumor tissue still remains a major obstacle for RCVs. Recent understanding in the cancer biology may be exploited to design tumor-selective viral replication to restrict its lytic effects to cancer cells. Dysregulation of the normal control over cell cycle and the circumvention of physiological apoptotic signals, may allow tumor-selective replication of an engineered virus, and subsequently, direct oncolysis by viral cell-killing.

Conditionally-replicative adenoviruses (CRAds) are designed either by deletion of adenoviral natural genes encoding cell-cycle regulatory proteins, or by placing a tissue-specific promoter to control a viral gene essential for replication. An example of the former is the deletion of CRAd E1A of amino acids 121–127, resulting in loss of its conserved region 2, thereby precluding its binding to Rb and eliminating the inhibitory effect of Rb on E2F.

Consequently, the engineered adenovirus is expected to replicate selectively within cells where the G1-S phase checkpoint is impaired, i.e. tumor cells [144,145]. CR2 deletion complemented by an additional aminoterminal deletion may be even more selective [146]. Similarly, deletion of the adenoviral E1B 55 k protein was suggested to be selective to p53-mutant cells [1], but has been questioned since. Despite the biological uncertainty, the E1B-deleted ONYX-015 virus showed a beneficial clinical effect in patients with recurrent head and neck carcinomas, and was selectively infecting the tumor cells [147]. Since ONYX-015 does not have a therapeutic transgene but relies on its lytic effect, this has been the first clinical demonstration of CRAd utility for cancer. The other strategy for designing CRAds is the use of tissue-specific promoters to drive expression of E1A, thereby restricting viral replication to specific tissues or tumors. The application of heterologous promoters in adenovirus vectors is difficult because their activity and specificity is often affected by viral enhancers and promoters present in the vector genome.

Nonetheless, E1A gene expressed from the alpha-fetoprotein (αFP) gene promoter can induce relatively selective replication in hepatocellular carcinoma cells and SQ xenograft tumors [148].

Tight control of adenoviral E1A expression under the minimal PSA enhancer/promoter can confer prostate-specific oncolytic effect [149]. The first

generation prostate-specific CRAd replicates in PSA-positive prostate carcinoma cell lines but not in PSA-negative human cell lines, including other prostate cancer cell lines. The second generation vector is comprised of double prostate-specific control over expression of E1A and E1B (with PSA and kallikrein promoters, respectively), and has a better lysis profile following the reintroduction of the E3 region [2]. Furthermore, synergy of replication-dependent cytotoxicity with the chemotherapeutic agents paclitaxel (Taxol) or docetaxel (Taxotere) was achieved both in vitro and in vivo [150].

CRAds for breast carcinomas were also created whereby the DF3/MUC1 promoter, aberrantly activated in human breast carcinomas, was used to drive expression of E1A. This CRAd selectively replicates in MUC1-positive breast cancer cells and can inhibit human breast tumor xenografts in nude mice. Unlike its replication-deficient counterpart, the DF3/MUC1 CRAd replicated throughout the tumor xenografts. A transgene expressing version of this CRAd (armed CRAd), whereby the CMV promoter drove expression of TNF-α was associated with selective replication and production of TNF-α in cells expressing MUC1. Moreover, treatment of MUC1-positive, but not MUC1-negative, xenografts with a single injection of the armed CRAd was effective in inducing stable tumor regression [151]. Another approach to direct CRAd replication to estrogen receptors (ER)-positive breast cancers is based on replacing the E1a and E4 promoters by a portion of the pS2 promoter containing two estrogen-responsive elements (EREs). This promoter induces transcriptional activation of the E1a and E4 units in response to estrogens in cells that express ER. This CRAd is able to kill ER-positive human breast cancer cell lines as efficiently as the wild-type virus, with decreased capacity to affect ER-negative cells. Interestingly, by transcomplementation of E1a protein this CRAd allows the restricted replication of a conventional E1a-deleted adenoviral vector [152].

Lung cancer has also been addressed by a CRAd vector. KD1-SPB was designed with two safety features, namely E1A mutations and a lung-specific surfactant protein B (SPB) promoter replacing the E4 promoter. KD1-SPB replication was shown to be specific to papillary lung carcinoma cell line where the SPB promoter is active and could induce moderate but specific tumor inhibition in vivo [99].

A totally different strategy recently reported employs the generation of a functional promoter/gene constellation only upon adenoviral DNA replication, thereby suggesting selective transcriptional activation [153] (Table 4). This approach to discriminate between tumors and normal tissues is based on selective DNA replication of adenoviral vectors deleted of the entire E1 gene in tumor cells. Traditionally, E1 deletion is considered to abolish

Table 4
Transcriptional regulation of adenoviral replication

Genetic modification	Biological result	Therapeutic end-point	Reference
Deletion of E1A amino acids 121–127	Transformation deficiency	Restriction of viral replication to tumor cells	[144–146]
Deletion of E1B 55 K protein	Susceptibility to apoptosis	Restriction of viral replication to tumor cells	[1,147]
E1A control by αFP promoter	E1A transcription limited to αFP positive cells	HCC cell killing	[148]
E1A control by PSA promoter	E1A transcription limited to PSA positive cells	Prostate cancer cell killing	[149,150]
E1A control by DF3/MUC1 promoter	E1A transcription limited to DF3/MUC1 positive cells	Breast cancer cell killing	[151]
E1A control by pS2 promoter	E1A transcription limited to estrogen receptor positive cells	Breast cancer cell killing	[152]
E4 control by Sp-B promoter	E1A transcription limited to surfactant producing cells	Papillary lung cancer cell killing	[99]
E1 deletion	Selective DNA replication of adenoviral vectors in transcomplementing tumor cells	Liver metastases targeting	[153]

adenoviral replication. However, human tumor cell lines derived from colon, breast, cervical and lung cancers seem to support DNA replication of adenovirus deleted of E1 ($AdE1^-$). Inverted repeats (IRs) inserted into the E1 region of $AdE1^-$ vectors can mediate genomic rearrangements to bring a transgene into conjunction with the promoter. Thus, formation of a functional expression cassette depends on viral DNA replication which is expected to occur specifically in tumor cells. The cellular factors that trans-complemented $AdE1^-$ replication are uncertain, but the finding that the human papilloma virus (HPV) E6 and E7 proteins partially substitute for E1A/E1B proteins, may suggest that cellular transformation and progression to G1/S phase may circumvent the absolute requirement for E1 genes to support adenoviral replication.

6. Conclusions

Advances in molecular biology have enabled gene delivery into human somatic cells. These techniques have culminated in clinical gene therapy trials, including genetic disorders and cancer. While the former requires restoration of the function of defective genes, the latter employs cancer cell killing by cytoreductive, immunomodulatory, and anti-angiogenic means. Since these therapeutic effects are deleterious to normal tissues, cell-type specific targeting is essential. The use of tumor and tissue-specific transcription regulatory elements may both restrict transgene expression to malignant tissues and increase transgene expression and persistence in vivo. Since this is an essential requirement for safe and efficient gene therapy for human cancer, conditional gene expression is expected to be a pertinent part of cancer gene therapy. Additionally, because promoter- and tissue-specificity may determine host immune response to the transgene [154], the significance of transcriptional targeting is further underscored. As the processes of malignant transformation and tumor propagation continue to be understood at the molecular level, new therapeutic interventions will undoubtedly follow. Of these, transcriptional targeting of cancer cells holds a great promise to provide specific and efficient ways to treat cancer.

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References

[1] J.R. Bischoff, D.H. Kirn, A. Williams, C. Heise, S. Horn, M. Muna, L. Ng, J.A. Nye, A. Sampson-Johannes, A. Fattaey, F. McCormick, An adenovirus mutant that replicates selectively in p53-deficient human tumor cells, Science 274 (1996) 373–376.

[2] D.C. Yu, G.T. Sakamoto, D.R. Henderson, Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of calydon virus 764, an attenuated replication competent adenovirus for prostate cancer therapy, Cancer Res. 59 (1999) 1498–1504.

[3] F. Kanai, K.H. Lan, Y. Shiratori, T. Tanaka, M. Ohashi, T. Okudaira, Y. Yoshida, H. Wakimoto, H. Hamada, H. Nakabayashi, T. Tamaoki, M. Omata, In vivo gene therapy for alpha-fetoprotein-producing hepatocellular carcinoma by adenovirus-mediated transfer of cytosine deaminase gene, Cancer Res. 57 (1997) 461–465.

[4] W.P. Faulk, H. Harats, J.A. McIntyre, A. Berczi, I.L. Sun, F.L. Crane, Recent advances in cancer research: drug targeting without the use of monoclonal antibodies, Am. J. Reprod. Immunol. 21 (1989) 151–154.

[5] H.J. Haisma, J. Grill, D.T. Curiel, S. Hoogeland, V.W. van Beusechem, H.M. Pinedo, W.R. Gerritsen, Targeting of adenoviral vectors through a bispecific single-chain antibody [in process citation], Cancer Gene Ther. 7 (2000) 901–904.

[6] K.W. Peng, R. Vile, F.L. Cosset, S. Russell, Selective transduction of protease-rich tumors by matrix-metalloproteinase-targeted retroviral vectors, Gene Ther. 7 (1999) 1552–1557.

[7] W. Walther, U. Stein, Cell type specific and inducible promoters for vectors in gene therapy as an approach for cell targeting, J. Mol. Med. 74 (1996) 379–392.

[8] F. Bushman, Targeting retroviral integration [comment], Science 267 (1995) 1443–1444.

[9] T. Friedmann, J.K. Yee, Pseudotyped retroviral vectors for studies of human gene therapy, Nat. Med. 1 (1995) 275–277.

[10] N. Kasahara, A.M. Dozy, Y.W. Kan, Tissue-specific targeting of retroviral vectors through ligand-receptor interactions [see comments], Science 266 (1994) 1373–1376.

[11] J.L. Blackwell, C.R. Miller, J.T. Douglas, H. Li, G.E. Peters, W.R. Carroll, T.V. Strong, D.T. Curiel, Retargeting to EGFR enhances adenovirus infection efficiency of squamous cell carcinoma, Arch. Otolaryngol. Head Neck Surg. 125 (1999) 856–863.

[12] J.T. Nguyen, P. Wu, M.E. Clouse, L. Hlatky, E.F. Terwil-

liger, Adeno-associated virus-mediated delivery of antiangiogenic factors as an antitumor strategy, Cancer Res. 58 (1998) 5673–5677.
[13] N. Miller, J. Whelan, Progress in transcriptionally targeted and regulatable vectors for genetic therapy, Hum. Gene Ther. 8 (1997) 803–815.
[14] J.M. Sedivy, P.A. Sharp, Positive genetic selection for gene disruption in mammalian cells by homologous recombination, Proc. Natl. Acad. Sci. USA 86 (1989) 227–231.
[15] R.G. Vile, Tumor-specific gene expression, Semin. Cancer Biol. 5 (1994) 429–436.
[16] D.M. Nettelbeck, V. Jerome, R. Muller, Gene therapy: designer promoters for tumour targeting, Trends Genet. 16 (2000) 174–181.
[17] P.E. Koch, Z.S. Guo, S. Kagawa, J. Gu, J.A. Roth, B. Fang, Augmenting transgene expression from carcinoembryonic antigen (CEA) promoter via a GAL4 gene regulatory system, Mol. Ther. 3 (2001) 278–283.
[18] M. Takakura, S. Kyo, T. Kanaya, H. Hirano, J. Takeda, M. Yutsudo, M. Inoue, Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells, Cancer Res. 59 (1999) 551–557.
[19] J. Gu, S. Kagawa, M. Takakura, S. Kyo, M. Inoue, J.A. Roth, B. Fang, Tumor-specific transgene expression from the human telomerase reverse transcriptase promoter enables targeting of the therapeutic effects of the Bax gene to cancers, Cancer Res. 60 (2000) 5359–5364.
[20] R. Abdul-Ghani, P. Ohana, I. Matouk, S. Ayesh, B. Ayesh, M. Laster, O. Bibi, H. Giladi, K. Molnar-Kimber, M.A. Sughayer, N. de Groot, A. Hochberg, Use of transcriptional regulatory sequences of telomerase (hTER and hTERT) for selective killing of cancer cells, Mol. Ther. 2 (2000) 539–544.
[21] R.T. Jaggar, H.Y. Chan, A.L. Harris, R. Bicknell, Endothelial cell-specific expression of tumor necrosis factor-alpha from the KDR or E-selectin promoters following retroviral delivery, Hum. Gene Ther. 8 (1997) 2239–2247.
[22] B. Xie, N.N. Tam, S.W. Tsao, Y.C. Wong, Co-expression of vascular endothelial growth factor (VEGF) and its receptors (flk-1 and flt-1) in hormone-induced mammary cancer in the Noble rat, Br. J. Cancer. 81 (1999) 1335–1343.
[23] R. Heidenreich, A. Kappel, G. Breier, Tumor endothelium-specific transgene expression directed by vascular endothelial growth factor receptor-2 (Flk-1) promoter/enhancer sequences, Cancer Res. 60 (2000) 6142–6147.
[24] L.M. Ellis, Y. Takahashi, W. Liu, R.M. Shaheen, Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications, Oncologist 5 (2000) 11–15.
[25] U. Jager, Y. Zhao, C.D. Porter, Endothelial cell-specific transcriptional targeting from a hybrid long terminal repeat retrovirus vector containing human prepro-endothelin-1 promoter sequences, J. Virol. 73 (1999) 9702–9709.
[26] T. Walton, J.L. Wang, A. Ribas, S.H. Barsky, J. Economou, M. Nguyen, Endothelium-specific expression of an E-selectin promoter recombinant adenoviral vector, Anticancer Res. 18 (1998) 1357–1360.
[27] I. Vlodavsky, Y. Friedmann, M. Elkin, H. Aingorn, R. Atzmon, R. Ishai-Michaeli, M. Bitan, O. Pappo, T. Peretz, I. Michal, L. Spector, I. Pecker, Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis, Nat. Med. 5 (1999) 793–802.
[28] E.A. Clark, T.R. Golub, E.S. Lander, R.O. Hynes, Genomic analysis of metastasis reveals an essential role for RhoC, Nature 406 (2000) 532–535.
[29] C. Li, B. Guo, P.B. Wilson, A. Stewart, G. Byrne, N. Bundred, S. Kumar, Plasma levels of soluble CD105 correlate with metastasis in patients with breast cancer, Int. J. Cancer 89 (2000) 122–126.
[30] S. Kumar, A. Ghellal, C. Li, G. Byrne, N. Haboubi, J.M. Wang, N. Bundred, Breast carcinoma: vascular density determined using CD105 antibody correlates with tumor prognosis, Cancer Res. 59 (1999) 856–861.
[31] R. Henriksen, A. Gobl, E. Wilander, K. Oberg, K. Miyazono, K. Funa, Expression and prognostic significance of TGF-beta isotypes, latent TGF-beta 1 binding protein. TGF-beta type I and type II receptors, and endoglin in normal ovary and ovarian neoplasms, Lab. Invest. 73 (1995) 213–220.
[32] B. Bodey, B. Bodey Jr., S.E. Siegel, H.E. Kaiser, Upregulation of endoglin (CD105) expression during childhood brain tumor-related angiogenesis. Anti-angiogenic therapy, Anticancer Res. 18 (1998) 1485–1500.
[33] C.A. Brewer, J.J. Setterdahl, M.J. Li, J.M. Johnston, J.L. Mann, M.E. McAsey, Endoglin expression as a measure of microvessel density in cervical cancer, Obstet. Gynecol. 96 (2000) 224–228.
[34] E.A. Clark, J.S. Brugge, Integrins and signal transduction pathways: the road taken, Science 268 (1995) 233–239.
[35] S. Even-Ram, B. Uziely, P. Cohen, S. Grisaru-Granovsky, M. Maoz, Y. Ginzburg, R. Reich, I. Vlodavsky, R. Bar-Shavit, Thrombin receptor overexpression in malignant and physiological invasion processes, Nat. Med. 4 (1998) 909–914.
[36] A. Gougos, S. St Jacques, A. Greaves, P.J. O'Connell, A.J. d'Apice, H.J. Buhring, C. Bernabeu, J.A. van Mourik, M. Letarte, Identification of distinct epitopes of endoglin, an RGD-containing glycoprotein of endothelial cells, leukemic cells, and syncytiotrophoblasts, Int. Immunol. 4 (1992) 83–92.
[37] S.C. Even-Ram, M. Maoz, E. Pokroy, R. Reich, B.Z. Katz, P. Gutwein, P. Altevogt, R. Bar-Shavit, Tumor cell invasion is promoted by activation of protease activated receptor-1 in cooperation with the alpha vbeta 5 integrin, J. Biol. Chem. 276 (2001) 10952–10962.
[38] K. Kurohane, Y. Namba, N. Oku, Liposomes modified with a synthetic Arg-Gly-Asp mimetic inhibit lung metastasis of B16BL6 melanoma cells, Life Sci. 68 (2000) 273–281.
[39] D.P. Felsenfeld, D. Choquet, M.P. Sheetz, Ligand binding regulates the directed movement of beta1 integrins on fibroblasts, Nature 383 (1996) 438–440.
[40] L.H. Romer, N. McLean, C.E. Turner, K. Burridge, Tyrosine kinase activity, cytoskeletal organization, and motility in human vascular endothelial cells, Mol. Biol. Cell. 5 (1994) 349–361.

[41] T. Ishida, M. Ishida, J. Suero, M. Takahashi, B.C. Berk, Agonist-stimulated cytoskeletal reorganization and signal transduction at focal adhesions in vascular smooth muscle cells require c-Src, J. Clin. Invest. 103 (1999) 789–797.
[42] C.F. Waller, M. Ali, M. Heinzinger, W. Lange, Growth inhibition of Ph + progenitor cells from CML patients using the tyrosine kinase inhibitor CGP57148B, Anticancer Res. 20 (2000) 809–814.
[43] A. Garcia, A. Serrano, E. Abril, P. Jimenez, L.M. Real, J. Canton, F. Garrido, F. Ruiz-Cabello, Differential effect on U937 cell differentiation by targeting transcriptional factors implicated in tissue- or stage-specific induced integrin expression, Exp. Hematol. 27 (1999) 353–364.
[44] J.F. O'Rourke, G.U. Dachs, J.M. Gleadle, P.H. Maxwell, C.W. Pugh, I.J. Stratford, S.M. Wood, P.J. Ratcliffe, Hypoxia response elements, Oncol. Res. 9 (1997) 327–332.
[45] N. Koshikawa, K. Takenaga, M. Tagawa, S. Sakiyama, Therapeutic efficacy of the suicide gene driven by the promoter of vascular endothelial growth factor gene against hypoxic tumor cells, Cancer Res. 60 (2000) 2936–2941.
[46] A.L. Kung, S. Wang, J.M. Klco, W.G. Kaelin, D.M. Livingston, Suppression of tumor growth through disruption of hypoxia-inducible transcription, Nat. Med. 6 (2000) 1335–1340.
[47] G. Bosco, W. Du, T.L. Orr-Weaver, DNA replication control through interaction of E2F-RB and the origin recognition complex, Nat. Cell Biol. 3 (2001) 289–295.
[48] R. Morishita, G.H. Gibbons, M. Horiuchi, K.E. Ellison, M. Nakama, L. Zhang, Y. Kaneda, T. Ogihara, V.J. Dzau, A gene therapy strategy using a transcription factor decoy of the E2F binding site inhibits smooth muscle proliferation in vivo, Proc. Natl. Acad. Sci. USA 92 (1995) 5855–5859.
[49] M.J. Parr, Y. Manome, T. Tanaka, P. Wen, D.W. Kufe, W.G. Kaelin Jr., H.A. Fine, Tumor-selective transgene expression in vivo mediated by an E2F-responsive adenoviral vector, Nat. Med. 3 (1997) 1145–1149.
[50] P.H. Maxwell, G.U. Dachs, J.M. Gleadle, L.G. Nicholls, A.L. Harris, I.J. Stratford, O. Hankinson, C.W. Pugh, P.J. Ratcliffe, Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth, Proc. Natl. Acad. Sci. USA 94 (1997) 8104–8109.
[51] M.M. Katabi, H.L. Chan, S.E. Karp, G. Batist, Hexokinase type II: a novel tumor-specific promoter for gene-targeted therapy differentially expressed and regulated in human cancer cells, Hum. Gene Ther. 10 (1999) 155–164.
[52] J.D. Harris, A.A. Gutierrez, H.C. Hurst, K. Sikora, N.R. Lemoine, Gene therapy for cancer using tumour-specific prodrug activation, Gene Ther. 1 (1994) 170–175.
[53] T. Kumagai, Y. Tanio, T. Osaki, S. Hosoe, I. Tachibana, K. Ueno, T. Kijima, T. Horai, T. Kishimoto, Eradication of Myc-overexpressing small cell lung cancer cells transfected with herpes simplex virus thymidine kinase gene containing Myc-Max response elements, Cancer Res. 56 (1996) 354–358.
[54] A. Patterson, A.L. Harris, Molecular chemotherapy for breast cancer, Drugs Aging 14 (1999) 75–90.
[55] I. Chung, P.E. Schwartz, R.G. Crystal, G. Pizzorno, J. Leavitt, A.B. Deisseroth, Use of L-plastin promoter to develop an adenoviral system that confers transgene expression in ovarian cancer cells but not in normal mesothelial cells, Cancer Gene Ther. 6 (1999) 99–106.
[56] H. Sugimoto, N. Hashimoto, S. Ameshima, F. Gejyo, T. Ishizaki, S. Miyabo, The clinical study on secretory leukoprotease inhibitor (SLPI) in sera of patients with various pulmonary diseases, Rinsho Byori 43 (1995) 1267–1272.
[57] M. Nystrom, M. Bergenfeldt, I. Ljungcrantz, A. Lindeheim, K. Ohlsson, Production of secretory leucocyte protease inhibitor (SLPI) in human pancreatic beta-cells, Mediators Inflamm. 8 (1999) 147–151.
[58] S. van Wetering, A.C. van der Linden, M.A. van Sterkenburg, K.F. Rabe, J. Schalkwijk, P.S. Hiemstra, Regulation of secretory leukocyte proteinase inhibitor (SLPI) production by human bronchial epithelial cells: increase of cell-associated SLPI by neutrophil elastase, J. Invest. Med. 48 (2000) 359–366.
[59] R.I. Garver Jr., K.T. Goldsmith, B. Rodu, P.C. Hu, E.J. Sorscher, D.T. Curiel, Strategy for achieving selective killing of carcinomas, Gene Ther. 1 (1994) 46–50.
[60] H. Nguyen, L. Teskey, R. Lin, J. Hiscott, Identification of the secretory leukocyte protease inhibitor (SLPI) as a target of IRF-1 regulation, Oncogene 18 (1999) 5455–5463.
[61] Y. Wang, Q. Zhou, J. Liu, W. Zhang, H. Jing, L. Liu, J. Chen, Transcriptional expression of apoptosis suppression gene bcl-2 in non-small cell lung carcinoma, Zhonghua Yi Xue Yi Chuan Xue Za Zhi 17 (2000) 262–265.
[62] L. Dong, W. Wang, F. Wang, M. Stoner, J.C. Reed, M. Harigai, I. Samudio, M.P. Kladde, C. Vyhlidal, S. Safe, Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells, J. Biol. Chem. 274 (1999) 32099–32107.
[63] M.W. Mayo, C.Y. Wang, S.S. Drouin, L.V. Madrid, A.F. Marshall, J.C. Reed, B.E. Weissman, A.S. Baldwin, WT1 modulates apoptosis by transcriptionally upregulating the bcl-2 proto-oncogene, EMBO J. 18 (1999) 3990–4003.
[64] I. Sanchez-Garcia, D. Martin-Zanca, Regulation of Bcl-2 gene expression by BCR-ABL is mediated by Ras, J. Mol. Biol. 267 (1997) 225–228.
[65] Y. Wu, J.W. Mehew, C.A. Heckman, M. Arcinas, L.M. Boxer, Negative regulation of bcl-2 expression by p53 in hematopoietic cells, Oncogene 20 (2001) 240–251.
[66] J. Bange, E. Zwick, A. Ullrich, Molecular targets for breast cancer therapy and prevention, Nat. Med. 7 (2001) 548–552.
[67] C. Szczylik, T. Skorski, L. Malaguarnera, J. Hetman, S.T. Chen, B. Calabretta, Inhibition of in vitro proliferation of chronic myelogenous leukemia progenitor cells by c-myb antisense oligodeoxynucleotides, Folia Histochem. Cytobiol. 34 (1996) 129–134.
[68] T. Skorski, M. Nieborowska-Skorska, N.C. Nicolaides, C. Szczylik, P. Iversen, R.V. Iozzo, G. Zon, B. Calabretta, Suppression of Philadelphia1 leukemia cell growth in mice by BCR-ABL antisense oligodeoxynucleotide, Proc. Natl. Acad. Sci. USA 91 (1994) 4504–4508.
[69] T. Skorski, M. Nieborowska-Skorska, C. Szczylik, P. Kanakaraj, D. Perrotti, G. Zon, A. Gewirtz, B. Perussia, B.

Calabretta, C-RAF-1 serine/threonine kinase is required in BCR/ABL-dependent and normal hematopoiesis, Cancer Res. 55 (1995) 2275–2278.
[70] B. Calabretta, R.B. Sims, M. Valtieri, D. Caracciolo, C. Szczylik, D. Venturelli, M. Ratajczak, M. Beran, A.M. Gewirtz, Normal and leukemic hematopoietic cells manifest differential sensitivity to inhibitory effects of c-myb antisense oligodeoxynucleotides: an in vitro study relevant to bone marrow purging, Proc. Natl. Acad. Sci. USA 88 (1991) 2351–2355.
[71] P. de Fabritiis, M.C. Petti, E. Montefusco, M.S. De Propris, R. Sala, R. Bellucci, M. Mancini, A. Lisci, F. Bonetto, T. Geiser, B. Calabretta, F. Mandelli, BCR-ABL antisense oligodeoxynucleotide in vitro purging and autologous bone marrow transplantation for patients with chronic myelogenous leukemia in advanced phase, Blood 91 (1998) 3156–3162.
[72] F.E. Cotter, P. Johnson, P. Hall, C. Pocock, N. al Mahdi, J.K. Cowell, G. Morgan, Antisense oligonucleotides suppress B-cell lymphoma growth in a SCID-hu mouse model, Oncogene 9 (1994) 3049–3055.
[73] R.J. Klasa, M.B. Bally, R. Ng, J.H. Goldie, R.D. Gascoyne, F.M. Wong, Eradication of human non-Hodgkin's lymphoma in SCID mice by BCL-2 antisense oligonucleotides combined with low-dose cyclophosphamide [in process citation], Clin. Cancer Res. 6 (2000) 2492–2500.
[74] J. Trojan, T.R. Johnson, S.D. Rudin, B.K. Blossey, K.M. Kelley, A. Shevelev, F.W. Abdul-Karim, D.D. Anthony, M.L. Tykocinski, J. Ilan et al., Gene therapy of murine teratocarcinoma: separate functions for insulin-like growth factors I and II in immunogenicity and differentiation, Proc. Natl. Acad. Sci. USA 91 (1994) 6088–6092.
[75] H. Fakhrai, O. Dorigo, D.L. Shawler, H. Lin, D. Mercola, K.L. Black, I. Royston, R.E. Sobol, Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy, Proc. Natl. Acad. Sci. USA 93 (1996) 2909–2914.
[76] K.B. Cleutjens, H.A. van der Korput, C.C. van Eekelen, H.C. van Rooij, P.W. Faber, J. Trapman, An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter, Mol. Endocrinol. 11 (1997) 148–161.
[77] A. Gotoh, S.C. Ko, T. Shirakawa, J. Cheon, C. Kao, T. Miyamoto, T.A. Gardner, L.J. Ho, C.B. Cleutjens, J. Trapman, F.L. Graham, L.W. Chung, Development of prostate-specific antigen promoter-based gene therapy for androgen-independent human prostate cancer, J. Urol. 160 (1998) 220–229.
[78] Z. Culig, A. Hobisch, M.V. Cronauer, C. Radmayr, J. Trapman, A. Hittmair, G. Bartsch, H. Klocker, Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor, Cancer Res. 54 (1994) 5474–5478.
[79] J.P. Latham, P.F. Searle, V. Mautner, N.D. James, Prostate-specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector, Cancer Res. 60 (2000) 334–341.
[80] T. Segawa, H. Takebayashi, Y. Kakehi, O. Yoshida, S. Narumiya, A. Kakizuka, Prostate-specific amplification of expanded polyglutamine expression: a novel approach for cancer gene therapy, Cancer Res. 58 (1998) 2282–2287.
[81] R.G. Vile, I.R. Hart, In vitro and in vivo targeting of gene expression to melanoma cells, Cancer Res. 53 (1993) 962–967.
[82] A. Bateman, F. Bullough, S. Murphy, L. Emiliusen, D. Lavillette, F.L. Cosset, R. Cattaneo, S.J. Russell, R.G. Vile, Fusogenic membrane glycoproteins as a novel class of genes for the local and immune-mediated control of tumor growth, Cancer Res. 60 (2000) 1492–1497.
[83] W.M. Siders, P.J. Halloran, R.G. Fenton, Transcriptional targeting of recombinant adenoviruses to human and murine melanoma cells, Cancer Res. 56 (1996) 5638–5646.
[84] M. Ahonen, A.H. Baker, V.M. Kahari, High level expression of tissue inhibitors of metalloproteinases-1,-2 and -3 in melanoma cells achieved by adenovirus mediated gene transfer, Adv. Exp. Med. Biol. 451 (1998) 69–72.
[85] D. Grossman, P.J. Kim, J.S. Schechner, D.C. Altieri, Inhibition of melanoma tumor growth in vivo by survivin targeting, Proc. Natl. Acad. Sci. USA 98 (2001) 635–640.
[86] R.G. Vile, R.M. Diaz, N. Miller, S. Mitchell, A. Tuszyanski, S.J. Russell, Tissue-specific gene expression from Mo-MLV retroviral vectors with hybrid LTRs containing the murine tyrosinase enhancer/promoter, Virology 214 (1995) 307–313.
[87] K.P. Ponder, R.P. Dunbar, D.R. Wilson, G.J. Darlington, S.L. Woo, Evaluation of relative promoter strength in primary hepatocytes using optimized lipofection, Hum. Gene Ther. 2 (1991) 41–52.
[88] C.A. Richards, E.A. Austin, B.E. Huber, Transcriptional regulatory sequences of carcinoembryonic antigen: identification and use with cytosine deaminase for tumor-specific gene therapy, Hum. Gene Ther. 6 (1995) 881–893.
[89] T. Osaki, Y. Tanio, I. Tachibana, S. Hosoe, T. Kumagai, I. Kawase, S. Oikawa, T. Kishimoto, Gene therapy for carcinoembryonic antigen-producing human lung cancer cells by cell type-specific expression of herpes simplex virus thymidine kinase gene, Cancer Res. 54 (1994) 5258–5261.
[90] C.A. Richards, E.A. Austin, B.E. Huber, Transcriptional regulatory sequences of carcinoembryonic antigen: identification and use with cytosine deaminase for tumor-specific gene therapy, Hum. Gene Ther. 6 (1995) 881–893.
[91] J.D. Harris, A.A. Gutierrez, H.C. Hurst, K. Sikora, N.R. Lemoine, Gene therapy for cancer using tumour-specific prodrug activation, Gene Ther. 1 (1994) 170–175.
[92] K. Sikora, J. Harris, H. Hurst, N. Lemoine, Therapeutic strategies using c-erbB-2 promoter-controlled drug activation, Ann. N.Y. Acad. Sci. 716 (1994) 115–124, discussion 124-125, 140-143.
[93] J.M. DiMaio, B.M. Clary, D.F. Via, E. Coveney, T.N. Pappas, H.K. Lyerly, Directed enzyme pro-drug gene therapy for pancreatic cancer in vivo, Surgery 116 (1994) 205–213.
[94] M. Ohashi, F. Kanai, T. Tanaka, K.H. Lan, Y. Shiratori, Y. Komatsu, T. Kawabe, H. Yoshida, H. Hamada, M. Omata, In vivo adenovirus-mediated prodrug gene therapy for carcinoembryonic antigen-producing pancreatic cancer, Jpn. J. Cancer Res. 89 (1998) 457–462.

[95] M. Takeuchi, T. Shichinohe, N. Senmaru, M. Miyamoto, H. Fujita, M. Takimoto, S. Kondo, H. Katoh, N. Kuzumaki, The dominant negative H-ras mutant, N116Y, suppresses growth of metastatic human pancreatic cancer cells in the liver of nude mice, Gene Ther. 7 (2000) 518–526.

[96] R.P. Dematteo, S.J. McClane, K. Fisher, H. Yeh, G. Chu, C. Burke, S.E. Raper, Engineering tissue-specific expression of a recombinant adenovirus: selective transgene transcription in the pancreas using the amylase promoter, J. Surg. Res. 72 (1997) 155–161.

[97] M.J. Smith, M.D. Rousculp, K.T. Goldsmith, D.T. Curiel, R.I. Garver, Surfactant protein A-directed toxin gene kills lung cancer cells in vitro, Hum. Gene Ther. 5 (1994) 29–35.

[98] R.J. Bohinski, J.A. Huffman, J.A. Whitsett, D.L. Lattier, Cis-active elements controlling lung cell-specific expression of human pulmonary surfactant protein B gene, J. Biol. Chem. 268 (1993) 11160–11166.

[99] K. Doronin, M. Kuppuswamy, K. Toth, A.E. Tollefson, P. Krajcsi, V. Krougliak, W.S. Wold, Tissue-specific, tumor-selective, replication-competent adenovirus vector for cancer gene therapy, J. Virol. 75 (2001) 3314–3324.

[100] E. Morimoto, N. Inase, S. Mlyake, Y. Yoshizawa, Adenovirus-mediated suicide gene transfer to small cell lung carcinoma using a tumor-specific promoter, Anticancer Res. 21 (2001) 329–331.

[101] R. Newton, J. Seybold, L.M. Kuitert, M. Bergmann, P.J. Barnes, Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA, J. Biol. Chem. 273 (1998) 32312–32321.

[102] M. Yamamoto, R. Alemany, Y. Adachi, W.E. Grizzle, D.T. Curiel, Characterization of the cyclooxygenase-2 promoter in an adenoviral vector and its application for the mitigation of toxicity in suicide gene therapy of gastrointestinal cancers, Mol. Ther. 3 (2001) 385–394.

[103] J.M. Coulson, J. Stanley, P.J. Woll, Tumour-specific arginine vasopressin promoter activation in small cell lung cancer, Br. J. Cancer 80 (1999) 1935–1944.

[104] J.M. Coulson, C.E. Fiskerstrand, P.J. Woll, J.P. Quinn, E-box motifs within the human vasopressin gene promoter contribute to a major enhancer in small cell lung cancer, Biochem. J. 344 (3) (1999) 961–970.

[105] I.H. Maxwell, L.M. Glode, F. Maxwell, Expression of the diphtheria toxin A-chain coding sequence under the control of promoters and enhancers from immunoglobulin genes as a means of directing toxicity to B-lymphoid cells, Cancer Res. 51 (1991) 4299–4304.

[106] L.K. Venkatesh, M.Q. Arens, T. Subramanian, G. Chinnadurai, Selective induction of toxicity to human cells expressing human immunodeficiency virus type 1 Tat by a conditionally cytotoxic adenovirus vector, Proc. Natl. Acad. Sci. USA 87 (1990) 8746–8750.

[107] W.A. Marasco, S. Chen, J.H. Richardson, U. Ramstedt, S.D. Jones, Intracellular antibodies against HIV-1 envelope protein for AIDS gene therapy, Hum. Gene Ther. 9 (1998) 1627–1642.

[108] A.K. Fielding, M. Maurice, F.J. Morling, F.L. Cosset, S.J. Russell, Inverse targeting of retroviral vectors: selective gene transfer in a mixed population of hematopoietic and nonhematopoietic cells, Blood 91 (1998) 1802–1809.

[109] A. Grande, B. Piovani, A. Aiuti, S. Ottolenghi, F. Mavilio, G. Ferrari, Transcriptional targeting of retroviral vectors to the erythroblastic progeny of transduced hematopoietic stem cells, Blood 93 (1999) 3276–3285.

[110] M. Schmitt-Ney, B. Happ, P. Hofer, N.E. Hynes, B. Groner, Mammary gland-specific nuclear factor activity is positively regulated by lactogenic hormones and negatively by milk stasis, Mol. Endocrinol. 6 (1992) 1988–1997.

[111] W.H. Gunzburg, B. Salmons, Factors controlling the expression of mouse mammary tumour virus, Biochem. J. 283 (1992) 625–632.

[112] H.S. Pandha, L.A. Martin, A. Rigg, H.C. Hurst, G.W. Stamp, K. Sikora, N.R. Lemoine, Genetic prodrug activation therapy for breast cancer: a phase I clinical trial of erbB-2-directed suicide gene expression, J. Clin. Oncol. 17 (1999) 2180–2189.

[113] L.M. Anderson, S. Krotz, S.A. Weitzman, B. Thimmapaya, Breast cancer-specific expression of the *Candida albicans* cytosine deaminase gene using a transcriptional targeting approach, Cancer Gene Ther. 7 (2000) 845–852.

[114] N. Shi, W.M. Pardridge, Noninvasive gene targeting to the brain, Proc. Natl. Acad. Sci. USA 97 (2000) 7567–7572.

[115] J. Yoshida, M. Mizuno, K. Yagi, Efficient transfection of human interferon-beta gene to human glioma cells by means of cationic multilamellar liposomes coupled with a monoclonal antibody [corrected] [published erratum appears in J. Neurooncol. 1996 29 (2) 191], J. Neurooncol. 19 (1994) 269–274.

[116] M.R. Graf, M.R. Jadus, J.C. Hiserodt, H.T. Wepsic, G.A. Granger, Development of systemic immunity to glioblastoma multiforme using tumor cells genetically engineered to express the membrane-associated isoform of macrophage colony-stimulating factor, J. Immunol. 163 (1999) 5544–5551.

[117] J.R. Smith-Arica, A.E. Morelli, A.T. Larregina, J. Smith, P.R. Lowenstein, M.G. Castro, Cell-type-specific and regulatable transgenesis in the adult brain: adenovirus-encoded combined transcriptional targeting and inducible transgene expression, Mol. Ther. 2 (2000) 579–587.

[118] S.C. Ko, J. Cheon, C. Kao, A. Gotoh, T. Shirakawa, R.A. Sikes, G. Karsenty, L.W. Chung, Osteocalcin promoter-based toxic gene therapy for the treatment of osteosarcoma in experimental models, Cancer Res. 56 (1996) 4614–4619.

[119] T. Shirakawa, S.C. Ko, T.A. Gardner, J. Cheon, T. Miyamoto, A. Gotoh, L.W. Chung, C. Kao, In vivo suppression of osteosarcoma pulmonary metastasis with intravenous osteocalcin promoter-based toxic gene therapy, Cancer Gene Ther. 5 (1998) 274–280.

[120] G.S. Stein, J.B. Lian, J.L. Stein, A.J. van Wijnen, Bone tissue specific transcriptional control: options for targeting gene therapy to the skeleton, Cancer 88 (2000) 2899–2902.

[121] T.D. Southgate, S. Windeatt, J. Smith-Arica, C.A. Gerdes, M.J. Perone, I. Morris, J.R. Davis, D. Klatzmann, P.R.

Lowenstein, M.G. Castro, Transcriptional targeting to anterior pituitary lactotrophic cells using recombinant adenovirus vectors in vitro and in vivo in normal and estrogen/sulpiride-induced hyperplastic anterior pituitaries, Endocrinology 141 (2000) 3493–3505.

[122] T. Joki, M. Nakamura, T. Ohno, Activation of the radiosensitive EGR-1 promoter induces expression of the herpes simplex virus thymidine kinase gene and sensitivity of human glioma cells to ganciclovir, Hum Gene Ther. 6 (1995) 1507–1513.

[123] M.J. Staba, H.J. Mauceri, D.W. Kufe, D.E. Hallahan, R.R. Weichselbaum, Adenoviral TNF-alpha gene therapy and radiation damage tumor vasculature in a human malignant glioma xenograft, Gene Ther. 5 (1998) 293–300.

[124] G. Gazit, S.E. Kane, P. Nichols, A.S. Lee, Use of the stress-inducible grp78/BiP promoter in targeting high level gene expression in fibrosarcoma in vivo, Cancer Res. 55 (1995) 1660–1663.

[125] Y. Tsutsumi-Ishii, K. Tadokoro, F. Hanaoka, N. Tsuchida, Response of heat shock element within the human HSP70 promoter to mutated p53 genes, Cell Growth Differ. 6 (1995) 1–8.

[126] J. Zhu, B. Gao, J. Zhao, A. Balmain, Targeting gene expression to tumor cells with loss of wild-type p53 function, Cancer Gene Ther. 7 (2000) 4–12.

[127] R.V. Blackburn, S.S. Galoforo, P.M. Corry, Y.J. Lee, Adenoviral-mediated transfer of a heat-inducible double suicide gene into prostate carcinoma cells, Cancer Res. 58 (1998) 1358–1362.

[128] V. Braiden, A. Ohtsuru, Y. Kawashita, F. Miki, T. Sawada, M. Ito, Y. Cao, Y. Kaneda, T. Koji, S. Yamashita, Eradication of breast cancer xenografts by hyperthermic suicide gene therapy under the control of the heat shock protein promoter, Hum. Gene Ther. 11 (2000) 2453–2463.

[129] Q. Huang, J.K. Hu, F. Lohr, L. Zhang, R. Braun, J. Lanzen, J.B. Little, M.W. Dewhirst, C.Y. Li, Heat-induced gene expression as a novel targeted cancer gene therapy strategy, Cancer Res. 60 (2000) 3435–3439.

[130] M.C. Luna, A. Ferrario, S. Wong, A.M. Fisher, C.J. Gomer, Photodynamic therapy-mediated oxidative stress as a molecular switch for the temporal expression of genes ligated to the human heat shock promoter, Cancer Res. 60 (2000) 1637–1644.

[131] G. Gazit, G. Hung, X. Chen, W.F. Anderson, A.S. Lee, Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma, Cancer Res. 59 (1999) 3100–3106.

[132] W. Walther, U. Stein, I. Fichtner, M. Alexander, R.H. Shoemaker, P.M. Schlag, Mdr1 promoter-driven tumor necrosis factor-alpha expression for a chemotherapy-controllable combined in vivo gene therapy and chemotherapy of tumors [in process citation], Cancer Gene Ther. 7 (2000) 893–900.

[133] N.E. Vilaboa, A. Galan, A. Troyano, E. de Blas, P. Aller, Regulation of multidrug resistance 1 (MDR1)/P-glycoprotein gene expression and activity by heat-shock transcription factor 1 (HSF1) [in process citation], J. Biol. Chem. 275 (2000) 24970–24976.

[134] D.M. Harvey, C.T. Caskey, Inducible control of gene expression: prospects for gene therapy, Curr. Opin. Chem. Biol. 2 (1998) 512–518.

[135] H.H. Cha, E.J. Cram, E.C. Wang, A.J. Huang, H.G. Kasler, G.L. Firestone, Glucocorticoids stimulate p21 gene expression by targeting multiple transcriptional elements within a steroid responsive region of the p21waf1/cip1 promoter in rat hepatoma cells, J. Biol. Chem. 273 (1998) 1998–2007.

[136] M.O. Imhof, P. Chatellard, N. Mermod, A regulatory network for the efficient control of transgene expression, J. Gene Med. 2 (2000) 107–116.

[137] M.M. Burcin, B.W. O'Malley, S.Y. Tsai, A regulatory system for target gene expression, Front Biosci. 3 (1998) C1–7.

[138] R.V. Abruzzese, D. Godin, M. Burcin, V. Mehta, M. French, Y. Li, B.W. O'Malley, J.L. Nordstrom, Ligand-dependent regulation of plasmid-based transgene expression in vivo, Hum. Gene Ther. 10 (1999) 1499–1507.

[139] M.M. Burcin, G. Schiedner, S. Kochanek, S.Y. Tsai, B.W. O'Malley, Adenovirus-mediated regulable target gene expression in vivo, Proc. Natl. Acad. Sci. USA 96 (1999) 355–360.

[140] S. Takemoto, R. Trovato, A. Cereseto, C. Nicot, T. Kislyakova, L. Casareto, T. Waldmann, G. Torelli, G. Franchini, p53 stabilization and functional impairment in the absence of genetic mutation or the alteration of the p14(ARF)-MDM2 loop in ex vivo and cultured adult T-cell leukemia/lymphoma cells, Blood 95 (2000) 3939–3944.

[141] M.C. Coffey, J.E. Strong, P.A. Forsyth, P.W. Lee, Reovirus therapy of tumors with activated Ras pathway, Science 282 (1998) 1332–1334.

[142] S. Balachandran, G.N. Barber, Vesicular stomatitis virus (VSV) therapy of tumors, IUBMB Life. 50 (2000) 135–138.

[143] V. Krasnykh, I. Dmitriev, J.G. Navarro, N. Belousova, E. Kashentseva, J. Xiang, J.T. Douglas, D.T. Curiel, Advanced generation adenoviral vectors possess augmented gene transfer efficiency based upon coxsackie adenovirus receptor-independent cellular entry capacity, Cancer Res. 60 (2000) 6784–6787.

[144] C. Heise, T. Hermiston, L. Johnson, G. Brooks, A. Sampson-Johannes, A. Williams, L. Hawkins, D. Kirn, An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy, Nat. Med. 6 (2000) 1134–1139.

[145] K. Suzuki, J. Fueyo, V. Krasnykh, P.N. Reynolds, D.T. Curiel, R. Alemany, A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency, Clin. Cancer Res. 7 (2001) 120–126.

[146] J.A. Howe, G.W. Demers, D.E. Johnson, S.E. Neugebauer, S.T. Perry, M.T. Vaillancourt, B. Faha, Evaluation of E1-mutant adenoviruses as conditionally replicating agents for cancer therapy, Mol. Ther. 2 (2000) 485–495.

[147] F.R. Khuri, J. Nemunaitis, I. Ganly, J. Arseneau, I.F. Tannock, L. Romel, M. Gore, J. Ironside, R.H. MacDougall, C. Heise, B. Randlev, A.M. Gillenwater, P. Bruso, S.B. Kaye, W.K. Hong, D.H. Kirn, A controlled trial of

intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer [in process citation], Nat. Med. 6 (2000) 879–885.

[148] P.L. Hallenbeck, Y.N. Chang, C. Hay, D. Golightly, D. Stewart, J. Lin, S. Phipps, Y.L. Chiang, A novel tumor-specific replication-restricted adenoviral vector for gene therapy of hepatocellular carcinoma, Hum. Gene Ther. 10 (1999) 1721–1733.

[149] R. Rodriguez, E.R. Schuur, H.Y. Lim, G.A. Henderson, J.W. Simons, D.R. Henderson, Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells, Cancer Res. 57 (1997) 2559–2563.

[150] D.C. Yu, Y. Chen, J. Dilley, Y. Li, M. Embry, H. Zhang, N. Nguyen, P. Amin, J. Oh, D.R. Henderson, Antitumor synergy of CV787, a prostate cancer-specific adenovirus, and paclitaxel and docetaxel, Cancer Res. 61 (2001) 517–525.

[151] T. Kurihara, D.E. Brough, I. Kovesdi, D.W. Kufe, Selectivity of a replication-competent adenovirus for human breast carcinoma cells expressing the MUC1 antigen, J. Clin. Invest. 106 (2000) 763–771.

[152] R. Hernandez-Alcoceba, M. Pihalja, M.S. Wicha, M.F. Clarke, A novel, conditionally replicative adenovirus for the treatment of breast cancer that allows controlled replication of E1a-deleted adenoviral vectors, Hum. Gene Ther. 11 (2000) 2009–2024.

[153] D.S. Steinwaerder, C.A. Carlson, D.L. Otto, Z.Y. Li, S. Ni, A. Lieber, Tumor-specific gene expression in hepatic metastases by a replication-activated adenovirus vector, Nat. Med. 7 (2001) 240–243.

[154] L. Pastore, N. Morral, H. Zhou, R. Garcia, R.J. Parks, S. Kochanek, F.L. Graham, B. Lee, A.L. Beaudet, Use of a liver-specific promoter reduces immune response to the transgene in adenoviral vectors, Hum. Gene Ther. 10 (1999) 1773–1781.