Ovarian carcinoma is a leading cause of cancer death in women. Though advances in conventional therapies have been achieved, long-term survival rates for most patients diagnosed with ovarian cancer are still low. Therefore, novel molecular therapeutic strategies such as gene therapy are being intensively pursued. Such approaches are based on the enormous progress that has been achieved in the elucidation of the molecular foundations of ovarian cancer. In this regard transcriptional control elements (promoters) of genes frequently upregulated or specifically expressed in tumors can be applied in a heterologous context to drive expression of therapeutic genes in targeted gene therapy strategies. This review discusses transcriptional targeting strategies in ovarian cancer gene therapy and gives an overview of tumor-specific promoters (TSPs) that have been applied for this purpose.

Key Words: transcriptional targeting; tumor-specific promoters; ovarian cancer; gene therapy.

INTRODUCTION

Ovarian cancer afflicts more than 25,000 women annually in the United States. Due to the lack of effective prevention and screening modalities, the majority of patients who are diagnosed with epithelial ovarian cancer present with advanced-stage disease [1]. Advances in surgical technique and chemotherapy have resulted in response rates that exceed 70%; however, most patients with advanced-stage ovarian cancer recur [2]. As such, the 5-year survival rate for patients diagnosed with advanced-stage ovarian cancer is approximately 15–30% and most patients ultimately succumb to their disease. These ominous statistics justify the search for effective new therapies, such as gene therapy, for patients afflicted with ovarian cancer.

There is a rational basis to pursue gene therapy as a novel paradigm for ovarian cancer. In this regard, it has become apparent that transformation of normal tissue leading to the emergence of a malignant phenotype results from the accumulation of a series of acquired genetic lesions that enhance tumor cell survival and proliferation; induce invasion, neovascularization, and metastasis; and inhibit immunological surveillance and apoptosis. These processes are becoming increasingly elucidated in the context of ovarian cancer and offer opportunities to be manipulated by genetic means for therapeutic gains. The majority of clinical cancer gene therapy trials to date have depended on compartmental cancer models due to current limitations in gene delivery systems. The ability to concentrate vector in the abdominal “container,” the ease of access to the abdominal cavity, and the usual confinement of ovarian cancer to the abdominal cavity have made ovarian cancer an ideal model to investigate a variety of gene therapy approaches.

EFFECTOR STRATEGIES IN CANCER GENE THERAPY

Current approaches to cancer gene therapy can be divided into four broad categories: (a) mutation compensation; (b) molecular chemotherapy; (c) genetic immunopotentiation; and (d) genetic modulation of resistance/sensitivity. Strategies to achieve mutation compensation are designed to rectify the molecular lesions in cancer cells responsible for malignant transformation. This can involve replacement of a defective tumor suppressor gene (such as BRCA1 or p53) [3, 4], ablation of a dominant oncogene (such as erbB-2) [5], or manipulation of genes (such as bcl-2 or bax) that control apoptosis [6, 7]. Molecular chemotherapy methods are designed to eradicate tumor cells by the selective delivery or expression of a gene encoding a prodrug-converting enzyme (“suicide gene”) and subsequent application of the corresponding prodrug, e.g., herpes simplex virus thymidine kinase (HSV-tk)/ganciclovir (GCV) or Escherichia coli cytosine deaminase (CD)/5-FC [8,
Due to the bystander effect—the killing of nontransduced neighboring cells, e.g., via lateral diffusion of the activated drug—quantitative transduction of target cells is not necessary in the latter approach. Genetic immunopotentiation is defined as the introduction of genetic modifications into host or cancer cells to augment the immunologically mediated destruction of tumor cells [10]. Finally, investigators have used a variety of gene therapy strategies to alter conventional chemotherapy and radiation therapy resistance and sensitivity in both normal and cancerous tissues to enhance the therapeutic index [11, 12].

RATIONALE FOR TRANSCRIPTIONAL TARGETING IN CANCER GENE THERAPY

Clinical trials have confirmed the safety and feasibility of a variety of the gene therapy approaches in the context of ovarian cancer [3, 13–21]. Commonly employed vector systems used to deliver therapeutic genes to tumor cells include retroviruses, adenoviruses, adeno-associated viruses, and liposomes [22]. However, correlative laboratory studies have demonstrated the limited ability of current generation vector systems to efficiently transduce ovarian tumor cells [14, 17]. In addition, limitations in vector specificity can lead to transduction of normal cells and untoward toxicity, even in the setting of compartmental dosing [23]. Thus, vector optimization is critical for the derivation of efficient clinical gene therapeutic regimens for ovarian cancer.

A central problem of conventional systemic cancer therapies is their lack of specificity for tumor cells, resulting in serious side effects and dose limitations below efficiency of the regimen to cure disease. The success of gene therapy, as a new approach for cancer treatment, depends on its ability to achieve a high therapeutic index. Adverse effects due to gene expression in nontarget cells, most abundantly liver toxicity and toxicity to the bone marrow, have been reported in preclinical studies. Furthermore, ectopic gene expression in immune cells may be responsible for an immune response to the transgene, thus limiting therapeutic efficiency. Therefore, efficient gene therapy regimens require transgene expression in the tumor, which we have denoted as the “on” status, and absence of expression in the relevant normal tissues, which we have denoted as the “off” status.

Fortunately, recent efforts show that gene therapy offers the means to achieve tumor selective gene expression. Two strategies have been pursued to achieve this goal: Transductional targeting can be accomplished by modification of vector tropism [24]. This approach can result in infectivity enhancement of gene transfer vectors. For example, the extension of adenoviral vector binding to cellular integrins resulted in efficient transduction of primary ovarian cancer cells [25]. These often show low-level expression of the primary adenovirus receptor and are, thus, refractory to infection by wild-type adenovirus. Nevertheless, transductional targeting approaches resulting in specific gene transfer rather than infectivity enhancement are still limited. Alternatively, gene therapy targeting may be achieved by regulation of transgene expression, denoted as transcriptional targeting. Transcriptional targeting is of premier interest in tumor types for which specific cell surface markers are not described or do not exist. In this case, and for concepts like targeting of tumor physiology or therapy-induced gene expression (see below), transductional targeting is not possible. Transcriptional targeting will be of increasing importance as high efficient gene transfer vectors (see above: infectivity enhancement) and effector systems are developed. Furthermore, a combination of transcriptional and transductional targeting might increase the therapeutic index of corresponding gene therapeutic regimens.

THE CONCEPT OF TUMOR-SPECIFIC PROMOTERS

To date, vectors employed for cancer gene therapy have included constitutively active promoters such as the cytomegalovirus promoter (CMVp). CMVp is a strongly positive regulator but lacks expression specificity. An additional drawback of the CMVp derives from the fact that it represents a viral sequence and, thus, is frequently downregulated in vivo [26]. As a result, cellular promoters are being explored for cancer gene therapy aiming at specific and persistent expression of therapeutic genes in tumors [27, 28].

Most malignant cells retain the capacity to synthesize proteins that are specifically produced in the nonmalignant cell of origin. Several of these proteins, such as prostate-specific antigen, are upregulated in tumors. Other proteins are activated during tumorigenesis, whereby they are specifically expressed in tumor cells and prevalent among the diverse neoplastic cell populations. Gene regulatory elements that drive transcription of these proteins have the potential capacity to control gene expression in a tumor cell-specific manner. Additionally, promoters specifically active in cells of the tumor stroma, like tumor endothelial cells, or promoters induced by the unique tumor physiology, like hypoxia, show preferential activity in tumors. Transcriptional targeting is based on the use of these tissue or tumor-specific promoters (TSPs) in a heterologous context to direct the expression of therapeutic genes specifically to the tumor. A further strategy is to apply treatment-inducible promoters to express gene products that sensitize tumor cells to the inducing therapeutic regimen, resulting in a synergistic antitumor effect.

The elucidation of TSPs for cancer gene therapy requires definition of the promoter sequences responsible for specific activity. Therefore, deletion analyses of potential regulatory sequences are performed with reporter genes like GFP and LacZ (determination of the fraction of transduced cells) and luciferase (quantitation of promoter activity). For this purpose promoter sequences are cloned from genomic DNA, incorporated into the vector of choice, and transduced into target and nontarget cells. It should be noted that both activity and spec-
ifficacy of a candidate promoter might vary between different vectors. Promoter specificity is derived from the reporter activity in target versus nontarget cells relative to constructs with ubiquitous transcriptional control. Subsequent to reporter analysis, efficacy and toxicity studies are performed in vitro and in vivo, with constructs containing the candidate promoter driving a therapeutic gene. These studies result in the determination of the therapeutic index of the targeted construct.

Several promoters have been explored for gene therapy in a variety of cancer cell types. For example, the α-fetoprotein (AFP) promoter has been used to drive gene expression in hepatic carcinoma cells [29], the tyrosinase promoter in melanoma cells [30], the prostate specific antigen (PSA) promoter in prostate cancer cells [31], and the carcinoembryonic antigen (CEA) promoter in adenocarcinomas [32, 33]. The results of these studies have demonstrated the feasibility of using TSPs for targeting of cancer gene therapy in various cancer cell types. Most important, selective transcription and reduced side effects of TSP constructs compared with constructs with constitutive promoters have been shown in animal models [34]. Initial clinical gene therapy studies involving transcriptionally targeted gene expression are ongoing [35, 36].

**OVARIAN CANCER CELL-SPECIFIC PROMOTERS FOR GENE THERAPY**

There have been several candidate promoters analyzed in gene therapy studies for specific transcriptional control in ovarian cancer cells (for an overview see Table 1). For example, tumor-specific activation has been shown after infection with a retrovirus encoding the diphertheria toxin A chain gene under the control of the human chorionic gonadotropin promoter (hCGp). No expression of the severely toxic transgene was observed in normal ovarian cells and fibroblasts [37].

The secretory leukoprotease inhibitor (SLPI) gene has been shown to be expressed in ovarian carcinoma cells, as well as lung, breast, oropharyngeal, bladder, endometrial, cervical, and colorectal carcinomas. The SLPI promoter has been used in a plasmid construct to direct the expression of HSV-tk in a variety of carcinoma cell lines, including those of ovarian origin, i.e., SKOV3 cells, and has achieved specific cell killing [38, 39].

High-affinity folate receptors are expressed in normal ovaries, and in the vast majority of ovarian adenocarcinomas. The human α-folate receptor (HAFR) gene contains two tissuespecific promoters, P1 and P4. Goldsmith et al. constructed a recombinant adenovirus that harbored the P1 promoter driving the luciferase gene [40]. Several ovarian carcinoma cell lines were transduced, and correlation was demonstrated between folate receptor levels and reporter gene expression. A recombinant adenovirus is currently under construction with this promoter and the E. coli CD suicide gene.

The MUC1/DF3 gene encodes the polymorphic epithelial mucin (PEM), which is expressed in human glandular epithelium. This protein is overexpressed in most carcinomas, due mainly to transcriptional upregulation. The cancer-associated mucin, although very similar to its normal counterpart, has a distinct antigenic profile [41]. It has been reported that the epitope is expressed in ovarian adenocarcinomas, including serous, mucinous, endometrioid, clear cell, and undifferentiated subtypes [42]. Regulatory regions, with distal enhancer elements, have been identified [43]. Ring et al. generated recombinant retroviruses containing the HSV-tk gene under the control of the MUC1 promoter, and showed increased ganciclovir sensitivity in pancreatic and breast carcinoma cell lines [41]. Adenoviruses encoding the MUC1 promoter have achieved specific tumor gene expression in breast, pancreatic, and cholangiocarcinoma cells [44]. Finally, an adenovirus with MUC1 promoter driving the proapoptotic bax gene has shown specific cell killing in ovarian cancer cell lines and in a murine model of ovarian cancer [45].

**L-Plastin** is a member of the actin-binding proteins and is highly expressed in most human epithelial cancer cells [46]. The L-plastin promoter has been incorporated in a replication deficient adenovirus, driving the E. coli LacZ gene. This construct has been tested in a variety of cell lines, including ovarian cancer cells and mesothelial cells. The L-plastin-driven transgene expression appeared to be restricted to the ovarian carcinoma cells, while sparing the mesothelium [47]. Cytotox-
E-selectin  
Endothelium  
Highly expressed in OVCA; has been used in a retroviral vector driving TNF-
Cox-1  
Ovary  
Elevated expression in ovarian adenocarcinomas [104–108]  
Inhibin/activin  
uPA  
Many tumors UPA and its receptor are frequently elevated in OVCA [70, 102]; also, a radiation-
ET-1  
Midkine  
Many carcinomas [55, 56]  
Both are overexpressed in most OVCAs [63]  
UPA and its receptor are frequently elevated in OVCA [70, 102]; also, a radiation-
Cox-2  
Cyclin D1  
hTERT  
Carcinomas and germ cell tumors  
Telomerase is expressed in most OVCAs [96–100]; a plasmid construct driving caspase 8 gene has shown efficacy for cancer gene therapy [101]  
EGFR  
Carcinomas and germ cell tumors  
Frequently amplified or overexpressed in OVCA [52, 88–90]  
c-myc  
Cox-1  
Many carcinomas  
Expressed in some OVCAs [52, 86, 87]  
c-myc  
Many tumors  
Frequently amplified or overexpressed in OVCA [52, 88–90]  
Cyclin D1  
Many tumors  
Overexpressed or amplified in some OVCAs [91–95]  
hTERT  
Carcinomas and germ cell tumors  
Telomerase is expressed in most OVCAs [96–100]; a plasmid construct driving caspase 8 gene has shown efficacy for cancer gene therapy [101]  
EGFR  
Many tumors  
Frequently amplified or overexpressed in OVCA [52, 88–90]  
c-myc  
Many tumors  
Overexpressed or amplified in some OVCAs [91–95]  
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Telomerase is expressed in most OVCAs [96–100]; a plasmid construct driving caspase 8 gene has shown efficacy for cancer gene therapy [101]  
EGFR  
Many tumors  
Frequently amplified or overexpressed in OVCA [52, 88–90]  
c-myc  
Many tumors  
Overexpressed or amplified in some OVCAs [91–95]  
Endothelium  
Highly expressed in OVCA; has been used in a retroviral vector driving TNF-
Cox-1  
Ovary  
Elevated expression in ovarian adenocarcinomas [104–108]  

Opportunities for novel transcriptional targeting approaches may result from our increasing knowledge of the molecular biology of ovarian cancer, particularly in regard to overexpressed oncogenes and growth factors that accompany malignant transformation. The most well described oncogenes in ovarian cancer have been growth factor receptors of the erbB family and the cell signaling-related ras proteins [49, 50]. Additionally, alterations in expression of the epidermal growth factor receptor, the fibroblast growth factor receptor, PIK3, akt2, and fms have been identified in ovarian carcinoma cells [51, 52]. Finally, activity of telomerase, the polymerase that catalyzes the expansion of telomeres, has been detected in more than 90% of ovarian carcinomas [50, 53, 54]. Each of these overexpressed genes allows opportunities to take advantage of related promoters that would control transgene expression in normal and malignant cells (see Table 2).

Midkine (MK) is a heparin-binding, growth and differentiation factor, highly expressed in many malignant tumors. Specifically, the midkine gene has been shown to be overexpressed in most ovarian tumors [55]. Velculescu et al. performed serial analysis of gene expression (SAGE) of a variety of tumors versus their normal counterpart tissues, and midkine showed the second highest ratio of tumor/normal tissue expression [56]. An adenoviral vector with the MK promoter, upstream from the HSV-tk gene, has been injected intravenously into mice followed by GCV treatment. Lower liver toxicity was detected, resulting in a therapeutic index higher than that of a similar adenovirus encoding the CMV promoter [34].

CA125 antigen levels are elevated in 80 to 96% of epithelial tumors of the ovary [57]. Secretion of CA125 appears to be directly linked to the epithelial growth factor receptor signal transduction pathway [58]. However, its potential benefits for transcriptional targeting have not been investigated, as the gene has not yet been characterized [59]. Additionally, normal mesothelial cells have the capacity to secrete CA125 and may limit specificity of targeting [60, 61].

Other potential transcriptional targets include the hyaluronan receptor, which has been reported to be expressed in 40% of epithelial ovarian cancers [62]. Expression of endothelin 1 (ET-1) and endothelin A receptor (ET-AR) has been reported in more than 90 and 84% of ovarian cancer patients, respectively [63], and a plasmid encoding the ET-AR promoter and the luciferase reporter gene has been tested in Chinese hamster ovary cells [64]. Mesothelin is a surface glycoprotein that is expressed in ovarian cancers. However, it is constitutively expressed also in mesothelial cells, reducing its utility for ovarian transcriptional targeting [65–67].

The tumor-associated trypsin inhibitor, a potential marker for ovarian cancer, is expressed mainly in mucinous subtypes [68]. The matrix metalloprotease pump 1 (MMP7) is also frequently overexpressed in ovarian tumors [69]. Other genes, involved in tumor invasion and angiogenesis are being increas-
ingly elucidated in the context of ovarian cancer. For example, the urokinase-type plasminogen activator (uPA) and its receptor (uPAR), implicated in the cleavage of extracellular matrix proteins, are elevated in ovarian carcinoma cells [70].

The enzyme cyclooxygenase (Cox), which synthesizes prostaglandins from arachidonic acid, has two isoforms, the constitutively active Cox-1, and Cox-2, an inducible form associated with cellular growth and inflammatory processes. The Cox-2 protein has shown to be highly expressed in a number of epithelial tumors [71, 72]. Cox-1 has been proposed as a new ovarian cancer marker [73].

### TRANSCRIPTIONAL TARGETING OF TUMOR STROMA AND TUMOR PHYSIOLOGY: THERAPY-INDUCED TRANSCRIPTION

Gene expression can be transcriptionally targeted to ovarian cancer at several levels. First, therapeutic interventions can be guided through the recognition of molecular changes that are specific to the tumor cell. Second, the tumor vasculature can be targeted. This is an attractive substrate, as the endothelium is not prone to mutation-related resistance. In addition, neovascularization is a universal requirement for tumor growth, invasion, and metastasis. Third, promoters induced by physiological conditions unique to the tumor might be applied. Finally, treatment-responsive promoters can be constructed that demonstrate enhanced activity when exposed to conventional therapy (for an overview see Table 2).

The vascular endothelial growth factor (VEGF) and its receptors flt-1 and KDR are strongly expressed in most ovarian cancer tumors [74]. Thus, opportunities may exist for designing TSPs of genes controlling tumor angiogenesis in ovarian cancer. For instance, endothelial cell-specific expression of tumor necrosis factor α has been achieved after infection with retroviral constructs encoding the KDR or E-selectin promoter [75].

An alternative transcriptional strategy to target refractory tumor cells capitalizes on the use of promoters that are induced under certain conditions present in the tumor environment, (such as hypoxia) that hinder the efficacy of chemotherapy and radiotherapy. One such TSP is the promoter of the lactate dehydrogenase gene which is efficiently induced by the associated hypoxia-response enhancer (HRE) in ovarian cancer cell lines [76].

Several authors have identified specific molecular changes in ovarian cancer (i.e., increased 1q21 and 13q12 copy number) that correlate with resistance to chemotherapy. If these changes are present specifically in the tumor, and absent in the normal tissues, its molecular identification can drive gene therapy coadjuvant treatments toward refractory cancer cell subpopulations. For example, Vandier et al. have successfully used a plasmid encoding the metallothionein promoter to drive HSV-tk expression to kill ovarian carcinoma cisplatin resistant cells [77]. A retroviral vector, employing this promoter to express transforming growth factor β1 has been used in Chinese ovarian cancer cells [78]. Concomitant therapy may also modulate the activity of target promoters. We have observed, for example (data not published), that certain chemotherapy drugs can induce one promoter and shut off others. In the same way it has been reported that the mdr1 promoter can be induced after chemotherapy [79].

### TRANSCRIPTIONAL TARGETING OF VIRAL REPLICATION

In addition to regulating transgene expression, controlling replication of lytic viruses, including adenovirus and HSV, may also achieve the goals of tumor targeting in ovarian cancer gene therapy. In this new concept the transcriptionally targeted expression of essential viral genes results in an oncolytic virus specifically replicating in the tumor [80]. Recently, Zhang et al. have generated a conditionally replicative adenovirus by placing the EIA gene (necessary for viral replication) under the control of the L-plasmin promoter, resulting in tumor-specific replication competency [81].

### CONCLUSIONS AND OUTLOOK

Gene therapy is in its infancy with respect to its development as a therapeutic paradigm for ovarian cancer. Clinical trials over the past decade have begun to identify both the feasibility and the limitations of this approach. Incorporating the concept of transcriptional targeting will further refine the methodology, enhance the therapeutic index, and increase the applicability of gene therapy approaches for treatment of ovarian cancer.

New high-throughput differential display technologies [65, 82] and the recently completed sequencing of the human genome will result in the defining of a plethora of new target molecules for ovarian cancer. Furthermore, several recent studies focus on optimizing promoter elements or developing artificial promoters aiming at increased activity and specificity of transcriptional control for tumor targeting in gene therapy [27]. These new endeavors may result in the development of improved promoters with increased fidelity for ovarian cancer gene therapy.

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