MUC1 Promoter–Driven DTA as a Targeted Therapeutic Strategy against Pancreatic Cancer

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Abstract

Mucin1 (MUC1) is overexpressed in pancreatic ductal adenocarcinoma (PDA) and is associated with tumor aggressiveness, suggesting that MUC1 is a promising therapeutic target for promoter-driven diphtheria toxin A (DTA). Endogenous MUC1 transcript levels were analyzed by quantitative PCR (qPCR) in multiple PDA cells (Capan1, HPAFII, Su.86.86, Capan2, Hs766T, MiaPaCa2, and Panc1). Expression levels were correlated with luciferase activity and cell death after transfection with MUC1 promoter–driven luciferase and DTA constructs. MUC1-positive (+) cells had significantly elevated MUC1 mRNA expression compared with MUC1-negative (−) cells. Luciferase activity was significantly higher in MUC1+ cells when transfected with MUC1 promoter–driven luciferase and MUC1+ cells underwent enhanced cell death after transfection with a single dose of MUC1 promoter–driven DTA. IFNγ pretreatment enhanced MUC1 expression in MUC1− cells and induced sensitivity to MUC1–DTA therapy. Matched primary and metastatic tumor lesions from clinical specimens revealed similar MUC1 IHC labeling patterns, and a tissue microarray of human PDA biopsies revealed increased immunolabeling with a combination of MUC1 and mesothelin (MSLN) antibodies, compared with either antibody alone. Combining MUC1 with MSLN-targeted DTA enhanced drug efficacy in an in vitro model of heterogeneous PDA. These data demonstrate that MUC1 promoter–driven DTA preferentially kills MUC1-expressing PDA cells and drugs that enhance MUC1 expression sensitize PDA cells with low MUC1 expression.

Implications: MUC1 expression in primary and metastatic lesions provides a rationale for the development of a systemic MUC1 promoter–driven DTA therapy that may be further enhanced by combination with other promoter-driven DTA constructs. Mol Cancer Res; 13(3): 439–48. ©2014 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDA) is currently the fourth leading cause of cancer-related death in the United States, with approximately 45,000 new patients diagnosed per year (1). Cures are rarely achieved. Only 20% of patients present with localized and resectable disease (2), and even in this favorable group, the majority of patients recur. Systemic treatment options for PDA are limited and generally ineffective. Responses to conventional chemotherapy are uncommon and nearly all responses are temporary, due to acquired resistance by the tumor (3–5). Thus, there is a strong need to develop targeted therapies that are highly specific against individual pancreatic cancer cells and evade chemoresistance mechanisms. For PDA, this may involve lines of research that fall outside the boundaries of conventional pharmacologic strategies such as cytotoxic chemotherapy or targeted biologic therapies (e.g., antibodies) that inhibit important signaling pathways.

DNA delivery is one such promising therapy that offers new solutions to overcome obstacles encountered by conventional treatment strategies, including tumor heterogeneity, off-target drug effects, and redundancy in cell signaling pathways. One of the major technical limitations impeding the progress of DNA delivery as a viable treatment option for cancer to date has been poor systemic delivery and targeting specificity. However, there have been great advances in this area, and it is highly probable that safe and targeted systemic DNA delivery will be a viable treatment option for patients in the near future (6–8). For instance, biodegradable nanoparticle vectors have proven to be effective transfection agents, have demonstrated stability in the circulation, and are amenable to conjugation with targeting molecules that provide enhanced specificity (7). Therefore, there is a strong rationale to increase research efforts toward optimizing genetic-based treatment strategies against cancer.

Promoter-driven diphtheria toxin A (DTA) cancer treatment is particularly appealing because of the molecule’s potency. Diphtheria toxin is a 62,000 dalton protein that is naturally produced by the bacterium, Corynebacterium diphtheria, and is comprised of...
two fragment peptides. The B peptide (DTB) mediates toxin entry into susceptible cells, whereas the A toxin (DTA) executes cell killing within the cell. DTA catalyzes the transfer of ADP from NAD+ to eukaryotic polypeptide elongation factor (E2F), and in turn, inhibits protein synthesis. Remarkably, a single molecule of DTA is lethal to a cell in some model systems (9), which may minimize the emergence of DTA-specific resistance in cancer cells. We and others have developed a strategy of DTA therapy against pancreatic cancer using a pancreatic cancer–specific promoter to drive DTA expression (10–12). We generated a DNA construct with the mesothelin (MSLN) promoter linked to the DTA coding sequence, and used a highly efficient and biodegradable polymer (C32-117) to deliver DNA to pancreatic cancer cells. MSLN-positive pancreatic cancer cell lines were susceptible to treatment, whereas MSLN-negative cell lines were not (10). Although these data were encouraging, MSLN is diffusely expressed in just one sixth of pancreatic cancers (13), highlighting the need to develop additional cancer-specific promoters that (i) target a greater proportion of pancreatic cancers, (ii) target the most lethal pancreatic cancers or cell populations within a given tumor, and (iv) can be combined with MSLN promoter–driven DTA (and other promoter-driven constructs) in a multitargeted therapeutic approach required to overcome tumor heterogeneity.

A recent analysis of resected pancreatic cancers from short-term patients who died within 1 year of resection and long-term survivors (>30 month survival) was used to identify candidate promoters for DTA DNA therapy. A tissue microarray (TMA) comprised of 137 pancreatic cancers resected from patients in the two extreme survival groups was analyzed by IHC using antibodies against 13 of the most widely studied pancreatic cancer biomarkers (14). Expression patterns in 11 of the putative biomarkers were actually similar between survival groups, whereas MSLN and Mucin 1 (MUC1) expressions were far more predictive of early cancer-specific mortality than conventional pathologic features (e.g., positive lymph nodes, large tumor size, poor histology, or positive resection margins). Not only was MUC1 the most robust predictor of aggressive pancreatic cancer biology, it was also the most frequently expressed protein in pancreatic cancer out of the analyzed biomarkers (MUC1 was overexpressed in two thirds of PDAs (13).

Thus, MUC1 meets the key criteria for DTA promoter–driven therapy: It is expressed at low levels in normal cells (15–17), it is overexpressed in pancreatic cancer (particularly in the most aggressive pancreatic cancer subpopulations associated with early cancer-specific mortality), and its expression is predominantly governed at the transcriptional level (highlighted in this study). Herein, we are the first to develop and report MUC1 promoter–driven DTA therapy in any cancer type, and demonstrate targeted specificity against MUC1-positive (+) pancreatic cancer cells. In addition, we establish a pharmacologic strategy to induce drug sensitivity in resistant MUC1-negative (−) PDA cells. Finally, we provide a rationale, based on an analysis of a large sample set of human pancreatic cancer tissues and complementary data, for a multitargeted DTA therapeutic strategy using a cocktail of different promoter-driven DTA constructs.

Materials and Methods

Cell lines

Su.86.86, MiaPaCa2, Panc1, Hs766T, Capan1, Capan2, and HPAFII cells lines were obtained from the ATCC. MiaPaCa2, Panc1, Hs766T, and Capan1 were cultured in DMEM supplemented with 10% FBS, 1% glucose, and 1% penicillin/streptomycin (Life Technologies). Su.86.86 cells were cultured in RPMI Medium (Life Technologies). Capan2 cells were cultured in McCoy’s 5A Modified Media and HPAFII cells were cultured in Minimum Essential Media (Life Technologies). Cells were passaged to maintain confluency between 30% and 90%. Mycoplasma surveillance was carried out routinely using a PCR detection method (LookOut Mycoplasma PCR Detection Kit; Sigma-Aldrich). All experiments were carried out between passages 5 and 10 after receipt from the ATCC.

RT-PCR and qPCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) and RNA quantified on a Nanodrop 1000 (Thermo Fisher Scientific). cDNA templates were generated using the First-Strand cDNA Kit (Affymetrix). MUC1 mRNA was quantified by quantitative PCR (qPCR) with TaqMan gene-expression mastermix and FAM-based probes (endogenous control: 18S) on an ABI 7500 Fast analyzer (Life Technologies). High MUC1-expressing cell lines are referred to as MUC1+ and low expressing cell lines as MUC1−.

DNA constructs

Promoter-driven plasmid constructs are indicated in this article with the gene promoter in front (e.g., pMUC1 refers to a plasmid with the MUC1 promoter), and the gene regulated by that promoter indicated after the promoter (e.g., pMUC1/luciferase refers to a MUC1 promoter–driven luciferase plasmid). CAG is a constitutively active, nonspecific regulatory sequence containing a cytomegalovirus (CMV) enhancer and chicken β-actin promoter. The pCAG/luciferase (Lac) and pCAG/eGFP constructs were generated from pEGFP (Clontech) as previously described (6). The MUC1 promoter is a 2874 bp sequence with function principally attributed to a 725 bp sequence at the 3′-end (18). MUC1 promoter constructs were amplified from a commercial pDRIVE–hMUC1 plasmid containing the 800 upstream base pairs (InvivoGen). The promoter was amplified using a forward primer 5′-GCT TCT CTA-3′ and reverse primer 5′-AGA TCT CCA TGG TGG TGA AAT G-3′, which contained BglII and BgIII restriction sites, respectively. The MUC1 promoter was substituted through a multistep cloning process for the MSLN promoter in a previously generated plasmid derived from a pgLI4 backbone (Promega; ref. 10). The resulting subcloning procedures yield pMUC1/luc, pMUC1/DTA, and pMUC1/XX (no coding sequence, negative control) plasmid constructs. pMUC1/eGFP was generated by amplifying GFP from pCAG/eGFP (19) using the forward primer 5′-TAA TAA GAT CTA TCC ACC GGT CGC CAC C-3′ and reverse primer 5′-GCT TCT CTA-3′ and the reverse primer 5′-GCT TCT CTA ACC CGC TTT ACT TGT ACA GC-3′, which contained BglII and XbaI sites, respectively. The ampiclon was then substituted for DTA from pMUC1/DTA.

Transfection of DNA constructs

Cells were seeded on 6-well plates with roughly 250,000 cells per well. At 24 hours, cells were washed with Opti-MEM reduced serum medium (Invitrogen) in preparation for transfection. Lipofectamine 2000 Transfection Reagent (Invitrogen) was added to 4 μg of the DNA construct and incubated for 20 minutes in 2.5 mL of Opti-MEM. Following incubation, the DNA–Lipofectamine mixture was added to each well. After 5 hours, transfected cells
were fed with 2 mL of growth medium and 0.5 mL of FBS. Transfection experiments were performed in triplicate.

**Luciferase expression**

Cells were harvested 48 hours after transfection for luciferase detection using the Luciferase Assay Kit and Reporter Lysis Buffer (Promega). Luciferase activity was measured by a Veritas Microplate Luminometer (Turner/Promega). Experimental arms included pMUC1/XX (negative control), pMUC1/Luc, and pCAG/Luc (positive control).

Luciferase activity was also used to measure toxicity of the pMUC1/DTA construct. Cells were cotransfected with pCAG/Luc as a measure of cell viability, and either pMUC1/DTA or pMUC1/XX. The cells were collected and prepared for luciferase measurements after 48 hours.

**Cell survival assays**

Additional assays to estimate cell viability included PicoGreen and Trypan blue exclusion. For the former, cells were seeded at 50,000 cells per well in 24-well plates and incubated at 37°C overnight. The cells were transfected the next morning using Lipofectamine 2000 and DNA constructs. Each plate was seeded with a specific cell line and treated with pMUC1/XX or pMUC1/DTA using increasing amounts of DNA (dose response), and grown to high confluency over 5 to 6 days. After reaching confluency, cells were washed with PBS. Lysis was performed using denized water at 37°C for 1 hour. Following lysis, 100 µL of 0.05% Quant-IT PicoGreen dsDNA reagent (Invitrogen) was added to each well for 1 hour. Fluorescence of dsDNA was measured using a microplate reader (TECAN, Phoenix) as an estimate of cell proliferation (20). For Trypan blue exclusion (Invitrogen) assays, cells were counted 48 hours after transfections.

**Immunofluorescence**

Capan1 cells (as representative of a MUC1+ cell line) and Su.86.86 cells (as representative of a MUC1− cell line) were plated into 4-well chambers. The following day, Alexa Fluor 488 (Life Technologies) fluorescently labeled antibodies (MUC1, VU4H5, Santa Cruz Biotechnology; and MSLN, M285, Santa Cruz Biotechnology) were added to each well and cells were incubated overnight at 4°C. At 24 hours, the cells were washed and analyzed by microscopy (Zeiss).

**Immunohistochemistry**

Human pancreatic cancer samples from the Johns Hopkins Gastrointestinal Cancer Rapid Medical Donation Program (GICRMDP) were used to study MUC1 expression in matched pancreatic primary tumor and liver metastases (21). Paraffin embedded histologic sections were labeled with a MUC1 antibody (1:100, VP-M655; Vector Laboratories). As a separate experiment, a TMA consisting of 107 primary PDAs was immunolabeled using the same MUC1 antibody, MSLN antibody (1:100, VP-M649; Vector Laboratories), or both together (double antibody labeling). IHC was performed as previously described using heat-induced epitope retrieval with the Ventana BenchMark Ultra platform (13). Ventana ultraview kits were used for antibody detection (Ventana). All immunolabeled slides were scored using a 4-tiered IHC score based on the percentage of labeled neoplastic cells: 0 (0%–10%); 1 (11 to 25%); 2 (26%–75%); 3 (76%–100%), with scores of 2 or 3 categorized as high expression and scores of 0 or 1 categorized as low expression. IHC scores were determined by expert pancreatic cancer pathologists (A.K. Wiltkie-Wicz, W. Jiang, and C.A. Iacobuzio-Donahue).

**Statistical analysis**

Cell survival analyses performed by Trypan blue exclusion or luciferase detection were compared using the two-tailed Student t test. RNA expression was similarly analyzed by the t test, using C values as an estimate of expression. IHC scores were compared between paired primary and metastases using the Spearman rank-order correlation coefficient (http://vassarstats.net/corr_rank.html). Immunolabeling patterns between antibodies were tested with the χ2 test (Stata 12.0). Scatter plots were analyzed by performing a regression analysis of regression coefficients of trends (Stata 12.0). Actual doses are plotted on the x-axis for clarity in provided graphs, but log10 dose was used for statistical analyses. Error bars included in histograms are reported as SDs. Significance was accepted for P < 0.05.

**Results**

**Profiling MUC1 mRNA and promoter levels in pancreatic cancer cell lines**

MUC1 mRNA levels were assessed in a panel of seven pancreatic cancer cell lines to identify MUC1+ and MUC1− cell lines. Capan1, Capan2, and HPAFII were identified as high expressing cell lines using an arbitrary cutoff of at least 5-fold compared with the Su.86.86 cell line. MiaPaCa2, Hs766T, Panc1, and Su.86.86 were identified as low expressing cell lines (P < 0.001, Fig. 1A). These data are consistent with prior studies (22–28).

To validate MUC1 promoter–transcriptional activity, we used a pMUC1/Luc reporter construct (10). Luciferase activity was measured 48 hours after cell transfections and correlated with endogenous MUC1 mRNA expression (Fig. 1B), indicating that MUC1 expression is predominantly regulated at the transcription level and that MUC1 promoter–driven constructs exhibit specificity for MUC1+. Fluorescence microscopy after transfections with pMUC1/eGFP validated these findings (data not shown).

**pMUC1/DTA toxicity is specific for MUC1+ pancreatic cancer cells**

We assessed transfection efficiency in our experimental model by transfecting MUC1+ (Capan1) and MUC1− (SU.86.86) cell lines with pCAG/eGFP DNA (GFP regulated by a constitutively active promoter). These data reveal a transient transfection efficiency between 10% and 17% across cell lines (Fig. 2A).

To estimate lethality of pMUC1/DTA, transfections (pMUC1/DTA and pMUC1/XX) were performed in 96-well plates with increasing amounts of plasmid, and PicoGreen measurements of DNA content were performed as an estimate of cell proliferation (29). Transfection experiments were performed in all four MUC1+ cell lines (Su.86.86, MiaPaCa2, Panc1, and Hs766T) as well as the three MUC1− cell lines (Capan1, Capan2, and HPAFII). A significant increase in cell death (based on the decrease in dsDNA compared with the negative control plasmid) was only observed for MUC1+ cells. Estimated cell death ranged between 65% and 85% for the MUC1− cells, and 10% and 35% for MUC1+ cells (Fig. 2B–H).

As a separate survival assay (10), luciferase activity was measured after cotransfection of cells with pMUC1/DTA and pCAG/Luc (CAG is a strong constitutive promoter that induces luciferase expression in all living cells transfected with the construct). A reduction in luciferase activity in this assay correlates directly with
MUC1 expression across pancreatic cancer cell lines. A, MUC1 mRNA levels measured by qPCR. B, luciferase expression after transfection with pMUC1/luciferase. 
P values for both figures represent comparisons between the means of MUC1+ cell lines and MUC1− cell lines.

Figure 1.

MUC1 expression in primary PDA correlates with MUC1 expression in patient-matched liver metastases

An effective therapy (e.g., pMUC1/DTA) against PDA in patients is contingent on effective systemic activity against both primary and metastatic lesions because distant disease is virtually always present at presentation (micrometastases are the norm even for patients with localized disease; ref. 34). Therefore, immunohistochemical staining to assess MUC1 protein expression was performed in 15 matched primary PDA and liver metastases from patient tissues included in a Rapid Autopsy study (21). MUC1 expression in the primary tumor was similar to MUC1 expression in metastases (Spearman’s rank correlation, \( \rho = 0.656, P = 0.007 \)), suggesting that treatments directed at the primary PDA tumor mass should also target systemic disease in those patients (Fig. 5).

A rationale for overcoming tumor heterogeneity: multipromoter approach to DTA therapy

Targeted (or personalized) therapy against an appealing oncogene or pathway may be effective in a susceptible cell culture model with homogeneous target expression, yet may be ineffective in heterogeneous patient tumors comprised of billions of cells with varied genetics and diverse microenvironments. We examined this hypothesis using a TMA of 107 resected PDAs. Tissues were labeled with antibodies against MUC1, MSLN, or both antibodies simultaneously. In this cohort, 56% had high MSLN expression (IHC score of 2 or 3) and 62% had high MUC1 expression. However, when both antibodies were used together (as a combination double-immunolabeling experiment), 82% of tumors had a high labeling pattern (Fig. 6A). These data provide evidence from primary patient tumors that a combination of promoter-driven therapies against two gene targets would likely achieve greater therapeutic coverage than just a single construct. In the microarray, 22% of tumors had a high IHC score (2 or 3) when
the combined antibody cocktail was tested, but a low score when just one of the two antibodies were used (i.e., not in combination), highlighting the subgroup of patients that would benefit the greatest from a cocktail of therapeutic DNA constructs. Only four PDAs (4.6%) had low immunolabeling with both antibodies together, but high immunolabeling when one of the antibodies was used (i.e., discordant results), indicating that the double-labeling IHC experiment was highly reproducible and accurate as an assay to assess simultaneously expression patterns of the two biomarkers. Figure 6B and C provide representative examples of tumors that label diffusely with one antibody, but not the other. In each case, labeling with both antibodies together revealed a more widespread expression pattern.

There are no established preclinical cancer models that accurately simulate the molecular heterogeneity present in human pancreatic cancer, because pancreatic cancer develops in patients slowly over 20 years (35). However, as a crude simulation of tumor heterogeneity, we mixed two different cell lines that differed in MSLN expression to demonstrate the utility of a multi-targeted promoter-driven DTA approach experimentally. Capan1 is MUC1+/MSLN+ and Hs766T is MUC1−/MSLN+ (Fig. 1; ref. 10). These expression profiles are illustrated by immunofluorescence shown in Fig. 6D. When the cell lines were mixed, MSLN labeling was homogeneous, whereas MUC1 labeling was incomplete. The cell lines were then mixed in varied proportions, and were treated with promoter-driven DTA constructs as shown.

Figure 2. Transfection efficiency and PicoGreen cell survival assays. A, GFP expression after transfection with pCAG/GFP in a MUC1+/MUC1− cell line. The percentage of GFP+ cells are graphed to the right. B to E, pMUC1/DTA therapy in cell lines with low MUC1 promoter activity. F to H, pMUC1/DTA therapy in cell lines with high MUC1 promoter activity. P values were calculated using a log10 dose scale and reflect a comparison of regression coefficients of the survival curves; NS, not significant.
The greatest degree of killing, estimated by Trypan blue exclusion, was achieved by combining pMUC1/DTA and pMSLN/DTA (p = 0.03). Notably, treatment with only pMUC1/DTA was largely ineffective, because only Capan1 cells (comprising a minority of cells in this experiment) are susceptible.

**Discussion**

Promoter-driven DNA delivery has been tested in a variety of tumor types using both viral (27, 36–38) and nonviral vectors (6, 10, 39). With the former type, coding sequences for metabolic enzymes are typically delivered (e.g., thymidine kinase or cytosine deaminase) to tumor cells, and these expressed enzymes activate prodrugs [ganciclovir (38) and 5-fluorocytosine (40), respectively]. Limitations of the viral/prodrug strategy include systemic toxicities of the prodrugs (because metabolic enzymes are also expressed in all normal cells), cell division is frequently required to achieve efficient cell death, and there are well-documented concerns regarding potential toxicity with viral therapy (41–45). Progress in systemic DNA delivery using nonviral nanoparticles (7, 8, 46–48), including ongoing studies by our group, suggests that this strategy offers a promising and perhaps safer alternative.

Although the future success of cancer gene therapy depends on continued progress in nonviral DNA delivery mechanisms, refinements in the design of DNA payloads represent an important initial step. To date, studies of DTA gene delivery in pancreatic cancer have been performed by our group using the MSLN promoter to drive DTA expression (10), as well as a group from Israel who have used promoters associated with IGF2 and H19 (12). To our knowledge, this study represents the first description of a MUC1 promoter–driven diphtheria toxin construct to treat any tumor type. The MUC1 promoter is particularly appealing due to its high expression in PDA. Moreover, diffuse MUC1 expression was more frequently observed in the most aggressive PDAs (13), which are typically resistant to conventional chemotherapy.

Herein, we demonstrate that MUC1+ cells had increased MUC1 promoter activity, as evidenced by enhanced luciferase expression after transfections with pMUC1/Luc. Indeed, transfection with pMUC1/DTA DNA preferentially kills MUC1-expressing PDA cells. The data suggest that incomplete cell death in MUC1+ cells was likely limited by transfection inefficiency, as opposed to...
intrinsic or acquired chemoresistance. Notably, commercial transfection reagents were used in this study to generate proof-of-principle data, but superior DNA delivery systems exist (6, 49) to achieve higher DNA delivery. We also demonstrate that cotreatment with compounds that induce MUC1 expression (e.g., IFNγ) sensitize MUC1− cell lines to pMUC1/DTA therapy. In addition, an analysis of matched primary and metastatic tumors in patients revealed that MUC1-targeted therapy is promising as a systemic targeting strategy, because MUC1 expression observed in primary tumors is consistently expressed in metastatic deposits from the same patients.

Inter- and intratumoral heterogeneity is perhaps the greatest obstacle to effective cytotoxic or targeted therapy against PDA, yet this point is often overlooked in the literature. Numerous attempts to target critical oncogenes (e.g., KRAS) or signaling pathways in PDA have failed in patients (50–53), even after initial successes were observed in preclinical models or in patients harboring other tumor types (51). A possible explanation for these failures is that the biologic heterogeneity present in all PDAs (54, 55) enables resistant subpopulations to emerge, leading to tumor growth in the face of single-gene (or pathway) targeted therapies. This has been observed previously with targeted therapies used against mutated oncogenes in certain nonpancreatic cancers (such as gastrointestinal stromal cancers and lung adenocarcinomas), in which the emergence of subclones harboring secondary mutations leads to chemoresistance (56, 57). The same phenomenon likely occurs in PDA with conventional chemotherapy, because virtually all PDAs eventually develop resistance to standard-of-care cytotoxic therapies (3, 58).

Figure 4.
Sensitivity of MUC1+ PDA cells is not likely to be a function of acquired drug resistance. Immunofluorescence of MUC1− and MUC1+ cells using a MUC1 antibody before two successive rounds of pMUC1/DTA therapy. Cytotoxicity of pMUC1/DTA does not decrease in MUC1+ cells that survive a dose of pMUC1/DTA (average of three experiments).

Figure 5.
MUC1 expression is similar between paired primary PDA and liver metastases. Each number on the x-axis represents a separate patient set.
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Figure 6.
Multigene targeting covers more of the pancreatic cancer landscape than single-gene targeting. A, IHC of 107 PDAs with antibodies against MSLN, MUC1, or both; blue, high expression (IHC score of 2 or 3); red, low expression (IHC score of 0 or 1). B, representative PDA with high MUC1 expression, low MSLN expression, and MUC1/MSLN double immunolabeling. C, representative with low MUC1 expression, high MSLN expression, but high MUC1 and MSLN double immunolabeling. For both B and C, stromal tissue (non-neoplastic) comprises 50% to 80% of the sample and does not label positively for MUC1 and MSLN. D, immunofluorescence of MUC1 and MSLN in CAPAN1, H5766T, and a mixture of CAPAN1+H5766T cells. IMUC1 means incompletely MUC1 due to the contribution of H5766T to the mixture. E, a combination of pMUC1/DTA and pMSLN/DTA was more effective than either construct alone in an in vitro model of tumor heterogeneity, in which two cell lines are mixed in varied proportions. HS766T cells are MUC1+/MSLN−, whereas Capan1 cells are MUC1−/MSLN+. Live micrographs are presented on the left and the results of Trypan blue exclusion appear on the right. In all cases, each plasmid construct was given at a dose of 2 μg of DNA/experimental arm. Analyzed together, only the combination of DTA constructs was significantly different than the negative control (P = 0.03).

Although the problem of tumor heterogeneity is intuitive and well characterized in the cancer biology literature (59), studies have not adequately quantified tumoral heterogeneity in the context of targeted therapeutics. Here, we demonstrate that MUC1- and MSLN-targeted therapies should at least partially treat 60% of patients with PDAs when used alone. High protein expression was considered when the IHC score was 2 or greater (which is typical in the pathology literature; 13, 60), which means that positively labeled tumor may only have absent expression in almost three quarters of the neoplastic cells. Therefore, a sizeable proportion of unsusceptible cells may remain. It is easy to appreciate that targeted therapies against cancers with “high” expression would likely be ineffective in many instances. Simultaneous labeling of the TMA by MUC1 and MSLN antibodies increased the therapeutic coverage of the tumors substantially, suggesting that combining promoter-driven DTA therapies to target both cell populations would likely be more potent. This concept was further validated experimentally in an in vitro model of tumor heterogeneity, in which two cell lines with different gene-expression profiles were mixed. A combination of promoter-driven DTA therapies proved to be more potent than treatment with a single promoter–DTA construct.

Although the concept of a multidrug or targeted approach makes intuitive sense and is a standard practice in clinical oncology, promoter-driven DTA gene therapy is particularly conducive to this approach because the DNA constructs are so tunable (more so than conventional pharmacologic approaches). There is virtually no practical limitation to the number of promoter constructs that may be generated for cancer-specific targeting, using straightforward molecular cloning techniques. Along these lines, promoters that target the tumor microenvironment (>70% of the PDA tumor mass) may be designed to overcome barriers to drug delivery to neoplastic cells (61). This capability, unique to promoter-driven DTA therapy, provides a stark contrast with conventional pharmacologic approaches to treat PDA, in which no straightforward strategies exist to overcome drug resistance.

There remain technical challenges associated with systemic and tumor-targeted DNA delivery, such as off-target toxicity. For instance, certain subpopulations of normal cells (such as gastrointestinal and breast epithelial cells) express MUC1 (15, 62). It is, therefore, conceivable that pMUC1/DTA would cause gastrointestinal side effects in patients. However, the toxicity profile may merely resemble the effects of currently used cytotoxic chemotherapy, and therefore this potential limitation should not stand in the way of continued preclinical investigations. Importantly, several aspects of nanoparticle-delivered DTA gene therapy (currently under development by our group and others; refs. 8, 63) may be exploited to mitigate or eliminate these potential side effects. First, targeting moieties (e.g., antibodies, receptor ligands, peptides) specific for cancer cells may be used to enhance cancer specificity. Second, specificity can be further enhanced by requiring the activity of multiple promoters for DTA expression (e.g., a dual-promoter DTA construct), in which activity of the first promoter triggers recombination at a second promoter site on
the construct that is required for DTA expression (although this intervention may sacrifice therapeutic potency). Third, nanoparticles may be formulated with promoter-driven reporter systems (e.g., fluorescent, nuclear imaging capable, etc.), so that a test dose may be administered to patients before delivering promoter-driven cytotoxic (e.g., DTA) therapy to test drug targeting specificity in vivo. Our group is currently looking to capitalize on each of these nanoparticle enhancements to deliver MUC1 and MSLN promoter–driven DTA therapy systemically to target cancers with high potency and specificity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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Grant Support

This work was supported by PA11-297, 1 R21 CA173605-01A1, and IBCG-08-060-04 funded by the American Cancer Society.

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Received April 16, 2014; revised September 4, 2014; accepted September 25, 2014; published OnlineFirst October 21, 2014.

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Molecular Cancer Research

MUC1 Promoter–Driven DTA as a Targeted Therapeutic Strategy against Pancreatic Cancer


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