THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 25, Issue of June 21, pp. 22980–22984, 2002
Printed in U.S.A.

Evaluating the Specificity of Antisense Oligonucleotide Conjugates

A DNA ARRAY ANALYSIS*

Received for publication, April 8, 2002
Published, JBC Papers in Press, April 10, 2002, DOI 10.1074/jbc.M203347200

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Antisense oligonucleotides are potentially powerful tools for selective control of cellular and viral gene expression. Crucial to successful application of this approach is the specificity of the oligonucleotide for the chosen RNA target. Here we apply DNA array technology to examine the specificity of antisense oligonucleotide treatments. The molecules used in these studies consisted of phosphorothioate oligomers linked to the Antennapedia (Ant) delivery peptide. The antisense oligonucleotide component was complementary to a site flanking the AUG of the MDR1 message, which codes for P-glycoprotein, a membrane ATPase associated with multidrug resistance in tumor cells. Using a DNA array of 2059 genes, we analyzed cellular responses to molecules comprised of Ant peptide-oligonucleotide conjugates, as well as to the Ant peptide alone. Besides the expected reduction in MDR1 message level, 37 other genes (~2% of those tested) showed changes of comparable magnitude. The validity of the array results was confirmed for selected genes using Northern blots to assess messenger RNA levels. These results suggest that studies using antisense oligonucleotide technology to modulate gene expression need to be interpreted with caution.

Antisense oligonucleotides have proven to be powerful tools for selective regulation of gene expression in experimental settings and are currently being evaluated for their therapeutic potential in the clinic (1, 2). Crucial to both experimental and therapeutic applications of antisense is the issue of specificity. Although some studies have shown that antisense oligonucleotides can discriminate differences in target RNAs as small as a single base change (3), other studies have suggested that oligonucleotides can have biological effects that are not attributable to specific degradation or blockade of their target RNAs. These effects can be due to sequence-specific aptameric effects of oligonucleotides (4), to non-sequence-specific binding of oligonucleotides to proteins (5), or to RNA cleavage because of partial sequence matches (6). In addition, it is possible that the reagents used to deliver oligonucleotides to cells, including cationic liposomes (7, 8), polymers (9), or the type of delivery peptide used in this study (10) could also have effects on cellular processes that lead to changes in mRNA levels. To guard against these potentially artifactual effects, investigators in the field have largely adopted a set of standards and controls that must be met before claiming a specific antisense action (11). Although these criteria have been invaluable to this point, recent technological advances now permit even more stringent evaluation of the effects of antisense oligonucleotides.

In this report we have used DNA arrays to assess the selectivity of a set of antisense and control reagents that we have reported on previously (12). Thus we have synthesized and evaluated peptide-oligonucleotide conjugates comprised of an oligonucleotide sequence targeted to the AUG region of the MDR1 gene or its mismatch control. These were both conjugated to a 19-amino acid sequence (Ant), adapted from the antennapedia transcription factor, that is known to be useful for intracellular delivery of peptides and oligonucleotides (10). Cells were treated with the peptide-oligonucleotide conjugates, with the Ant peptide itself, or were maintained as untreated controls. Thereafter, message levels from 2059 genes were assessed using commercial, oligonucleotide-based DNA array technology (13, 14). In addition to the expected decrease in MDR1 message level, 37 additional genes displayed changes that were regarded as significant in comparing the three experimental conditions with the control situation.

MATERIALS AND METHODS

Cells—The multidrug-resistant cell line MES-SA/Dx5 was obtained from the ATCC. This line, originally obtained from uterine sarcoma fibroblasts, expresses high levels of MDR-1 mRNA and P-glycoprotein (15). The cells were grown in McCoy's medium containing 10% fetal calf serum and 60 ng/ml colchicine in an atmosphere of 95% air, 5% CO_2.

Peptide-Oligonucleotide Conjugate Synthesis—Peptide-oligonucleotide conjugates were prepared via disulfide bond formation. Specifically, phosphorothioate 20-mer anti-MDR1 5′-d(CCA-TCC-CGA-CCT-CGC-GCT-CC)-3′ and mismatch 5′-d(CCA-TAC-CAA-CAT-CAC-GCT-CC)-3′ oligonucleotides were conjugated with highly basic Ant peptide (NH2RQIKIWFQNRRMKWKKGGCCOOH), and the conjugates were purified by high pressure liquid chromatography as previously described (12). The conjugates also included a TAMRA (carboxylic acid of tetramethylrhodamine) fluorophore at the 3′-end. The 20-mer anti-MDR1 oligonucleotide was also used in unconjugated form in some studies below.

Treatment of Cells with Peptide-Oligonucleotide Conjugates—The experimental protocols were similar to those previously described (12). Briefly, MES-SA/Dx5 cells were grown in 162-mm flasks to 95% confluency and then seeded into 100-mm dishes at 2×10^6/dish in 10% fetal bovine serum (FBS)/McCoy's medium[1] and incubated for 24 h. The cells were washed twice with PBS (phosphate-buffered saline). The peptide-oligonucleotide conjugates or Ant peptide itself were diluted in 10% FBS/McCoy's medium to 0.5 μM and were added into the cells and incubated at 37 °C for 16 h; after a medium change, the cells were assayed 48 h later. This protocol was used for the DNA array, Northern blotting, and flow cytometry experiments described below.

* This work was supported by National Institutes of Health Grant PO1 GM59299. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: FBS, fetal bovine serum; PBS, phosphate-buffered saline; PKC, protein kinase C.

Scrape Loading of Antisense Oligonucleotides—We also used a scrape-loading procedure (16) to transiently disrupt cell membranes and allow direct loading of unconjugated oligonucleotides into MES-SA/Dx5 cells. Briefly, the cells were seeded 24 h before treatment in 100-mm dishes at 1.5×10^6 cells/dish in 3 ml of medium. For scrape loading the medium was replaced with 3 ml of growth medium containing 0.5 μM 20-mer anti-MDR1 oligonucleotide. Cells were then removed from the plate with a cell scraper (Costar, Corning, NY), replated in fresh medium, and assayed 48 h later.

Analysis of P-glycoprotein Levels—Cell surface expression of P-glycoprotein was determined using a flow cytometry assay as previously described (12). After treatment with the conjugates, cells were washed twice in PBS, trypsinized, and resuspended in 10% FBS/McCoy's medium. The cells were washed in PBS, and 50 μl of 20 μg/ml MRK16 anti-P-glycoprotein antibody (Kamiya, Thousand Oaks, CA) was added. After incubation for 45 min on ice, cells were washed three times in 10% FBS/PBS and then incubated 30 min with an FITC conjugated goat anti-mouse IgG (Sigma). After the incubation, the cells were washed twice in 10% FBS/PBS. The level of FITC fluorescence in viable cells (viability determined by light scatter) was quantitated using the Summit V3.0 software application (Cytomation Inc.) on a Becton Dickinson flow cytometer.

RNA Isolation—Cytoplasmic RNA was isolated from the cells using a kit according to a protocol suggested by the manufacturer (Qiagen Inc., Valencia, CA). The RNA concentration was measured by taking the $A_{260\ nm}$.

DNA Array Analysis—Array studies were conducted in a manner similar to those described elsewhere (17, 18). Isolated cytoplasmic RNA (0. 7 μg) was used to synthesize cDNA. A custom cDNA kit from Invitrogen was used with a T7-$(dT)_{24}$ primer for this reaction. Biotinylated cRNA was then generated from the cDNA reaction using the BioArray high yield RNA transcript kit (Affymetrix). The cRNA was then fragmented (5× fragmentation buffer: 200 mM Tris acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc) at 94 °C for 35 min before chip hybridization. Following the manufacturer's protocol, fragmented cRNA (15 μg) was added to the hybridization mixture. DNA arrays HC-G110 or HG-U95Av2 (Affymetrix) were hybridized for 16 h in a GeneChip Fluidics Station 400 and scanned with a Hewlett Packard GeneArray Scanner. Sample quality was assessed by examination of 3′ to 5′ intensity ratios of certain genes. Samples were normalized to the average hybridization intensity on each chip. All array studies were performed as three to six independent experiments. Affymetrix GeneChip Microarray Suite 4.0 software was used for the experimental protocol and for basic analysis. The Gene Spring 4.0.2 (Silicon Genetics) software package was used for additional data analysis.

Measurement of mRNA Levels—Northern blotting was done according to a standard protocol (19). Briefly, cytoplasmic RNA was isolated from the cultured cells according to a protocol suggested by the manufacturer (Qiagen Inc.). Five micrograms of RNA sample was resolved on a 0.8% agarose gel containing 1.2% formaldehyde and transferred into a nylon membrane, followed by UV cross-linking (Stratagene, La Jolla, CA). The blot was hybridized with ^{32}P-labeled human cDNA probes. The templates for the probes were gel-purified (gel purification kit, Qiagen Inc.) reverse transcription-PCR products of: human MDR1 with the forward primer 5′-ACC GCA ATG GAG GAG CAA AG-3′ and the reverse primer 5′-TTA AGC TCC CCA ACA TCG TG-3′; human PKCα with the forward primer 5′-CCT TCC AAC AAC CTT GAC C-3′ and the reverse primer 5′-TCG TGA CTC CAT CCA TCA TG-3′; leukocyte tyrosine kinase with the forward primer 5′-CCA TTC TCT GCT CTA GCC-3′ and the reverse primer 5′-GGG CAC AGG CAT TCA GCC-3′; β-actin with the forward primer 5′-CTT CCT TCC TGG GCA TGG A-3′ and the reverse primer 5′-AGG AGG AGC AAT GAT CTT GA-3′. The probes were synthesized by a random priming method using a commercial kit (Ambion, Inc., Austin, TX). The hybridized blot was washed twice with 2× SSC buffer at room temperature, followed by two washes of 2× SSC + 1% SDS at 60 °C and two washes of 0.1× SSC at room temperature. The blot was then exposed to Kodak film for 6–24 h prior to development.

RESULTS AND DISCUSSION

The effects of the Ant peptide-oligonucleotide conjugates in inhibiting expression of P-glycoprotein are illustrated in Fig. 1. Cell surface levels of P-glycoprotein were quantitated by flow cytometry, using an antibody that is directed to a P-glycoprotein epitope displayed on the external surface of cells, followed by a fluorescent second antibody (12). As expected based on previous studies (12), we observed substantial inhibition of

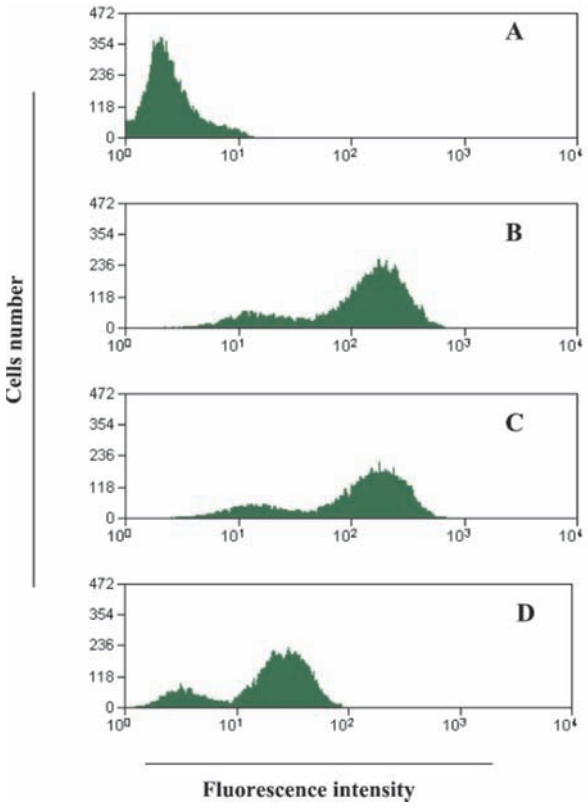

FIG. 1. **Antisense inhibition of P-glycoprotein expression measured by flow cytometry.** Approximately 10,000 MES-SA/Dx5 cells were counted in each sample. The abscissa represents the amount of bound fluorescent antibody. The ordinate is the number of cells at each level of fluorescence. *A*, unstained, untreated control (no primary antibody); *B–D*, samples immunostained with MRK16 anti-P-glycoprotein antibody; *B*, untreated control; *C*, Ant 20 mismatch treatment; *D*, antisense Ant 20 treatment. The bimodal shape of the peaks in *B–D* suggests some heterogeneity in P-glycoprotein expression in the original drug-resistant cell population.

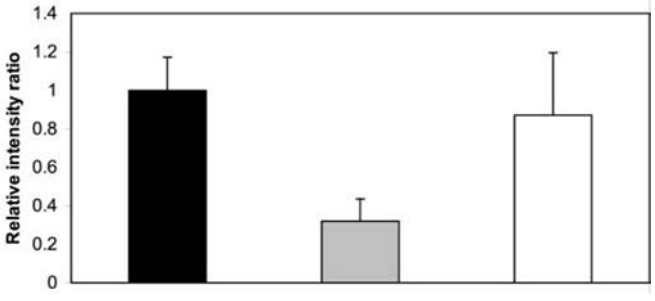

FIG. 2. **DNA array analysis of MDR1 gene expression.** The relative hybridization intensity is shown with the untreated control normalized to 1.0. *Black bar*, untreated control; *gray bar*, antisense Ant 20 treatment; *white bar*, Ant 20 mismatch treatment. The data represent the means ± S.E. of three to six independent experiments.

P-glycoprotein expression in cells treated with the antisense Ant 20 conjugate (Fig. 1*D*) and little effect in cells treated with the mismatch control conjugate (Fig. 1*C*, Ant 20 mismatch). Treatment with unconjugated antisense oligonucleotide or with the Ant peptide itself had no effect on P-glycoprotein levels (Ref. 12 and data not shown). The degree of inhibition of P-glycoprotein levels in the flow cytometry studies was ~85% for Ant 20 and 2% for Ant 20 mismatch *versus* untreated control.

To provide a broader perspective on the specificity of our peptide-oligonucleotide conjugates, we used Affymetrix DNA array technology to interrogate a set of 2059 cancer-related

TABLE I

Compilation of genes affected by peptide-oligonucleotide conjugates

The number of genes significantly affected by a particular treatment is shown. The criteria for attributing significance are discussed in the text.

Name	Number of genes affected	Genes increased	Genes decreased	Specific genes	Specific genes increased	Specific genes decreased
Genes affected by Ant 20	24	13	11	14	4	10
Genes affected by Ant 20 mismatch	20	12	8	10	3	7
Genes affected by Ant	8	6	2	4	2	2
Genes affected by both Ant 20 and Ant 20 mismatch	10	9	1			
Genes affected by Ant 20 and Ant 20 mismatch and Ant	4	4	0			

TABLE II

DNA array analysis of genes affected by peptide-oligonucleotide conjugates

The identity of the genes affected by peptide oligonucleotide conjugates and the approximate magnitude of the increase or decrease are indicated. *p* values (Student's *t* test) for treated *versus* untreated controls are shown. Genes whose message levels were affected by both anti-MDR1 peptide-oligonucleotide conjugate and by "free" anti-MDR1 oligonucleotide delivered by scrape loading are indicated with number symbols adjacent to the name of the gene. The data for the Ant 20 (antisense) conjugate represents six independent experiments. The data for the Ant 20 mismatch and the unconjugated Ant peptide represent three independent experiments.

Gene name	Increase (-fold change)	Decrease (-fold change)	*t* test (*p* value)	Affected by Ant 20	Affected by Ant 20 mismatch	Affected by Ant
Human *MEK5*	+(~3)		0.051	*	*	
Human tissue inhibitor of metalloproteinases-3	+(~3)		0.043	*	*	
Human PKC α	+(~2)		0.041	*	*	
Human stem cell leukemia	+(~2)		0.014	*		
Human heat shock factor 1 (*TCF5*)	+(~2)		0.048	*		
Human oncostatin-M specific receptor β	+(~2)		0.002	*		
DNA polymerase δ catalytic subunit #	+(~2)		0.018	*		
Human T-cell receptor active α-chain #	+(~2)		0.005	*	*	
Retinoic acid receptor, γ2	+(~2)		0.089	*	*	
Human *MDR1* #		**−(~3)**	**0.004**	*		
Human leukocyte tyrosine kinase		−(~3)	0.035	*		
Human DNA topoisomerase III		−(~3)	0.040	*	*	
Homo sapiens TGF-β type I receptor #		−(~3)	0.025	*		
Human *mdm2-E* (*mdm2*) #		−(~2)	0.032	*		
Integrin β3 (alternatively spliced) #		−(~2)	0.019	*		
Human interleukin-8 receptor type A #		−(~2)	0.008	*		
Human c-*erbA* #		−(~2)	0.109	*		
Human prolactin #		−(~2)	0.023	*		
Mitogen-induced nuclear orphan receptor #		−(~2)	0.047	*		
Homo sapiens vasopressin V3 receptor		−(~2)	0.015	*		
CD44 gene (cell surface glycoprotein CD44)	+(~3)		0.042		*	
Human transforming growth factor-β1	+(~2)		0.005		*	
Homo sapiens DNA ligase IV	+(~2)		0.045		*	
Homo sapiens transmembrane tyrosine kinase		−(~3)	0.016		*	
Human G protein-coupled receptor OGR1		−(~2)	0.080		*	
Ubiquitin-conjugating enzyme Ubch5		−(~2)	0.008		*	
Human TR3 orphan receptor		−(~2)	0.069		*	
Human cytochrome P450 (CYP2A13)		−(~2)	0.056		*	
Homo sapiens interleukin 1α (IL-1)		−(~2)	0.060		*	
Human Src-like adapter protein		−(~2)	0.030		*	
Human SH3 domain-containing protein	+(~3)		0.095			*
Human GTPase-activating protein (rap1GAP)	+(~2)		0.064			*
Granulocyte colony-stimulating factor receptor		−(~3)	0.020			*
Oncogene Aml1-Evi-1, fusion activated		−(~2)	0.009			*
Bcl-2 mRNA	+(~3)		0.011	*	*	*
Human C-C chemokine receptor type	+(~3)		0.015	*	*	*
Human protein kinase (JNK1)	+(~2)		0.001	*	*	*
Homo sapiens cadherin	+(~2)		0.036	*	*	*

genes. As seen in Fig. 2, the array data on the MDR1 gene agrees with the observations of Fig. 1 and shows a substantial reduction (about 3-fold) in MDR1 message expression in the cells treated with the Ant peptide-antisense conjugate but not in cells treated with the control mismatch conjugate. We next sought other genes whose message levels changed significantly from untreated control levels in response to one of the three experimental treatments. The following criteria were used to identify such genes: (*a*) there must be at least a 2-fold change in message level as compared with untreated control; (*b*) the hybridization intensity must be above 100 arbitrary units so as to exclude weak signals; (*c*) the standard deviation between experiments must be less than 100% of the relative intensity so as to exclude genes that did not change in a consistent manner in the several independent experiments. These are similar to criteria used in several other studies (13, 14). Based on these criteria, 38 genes including MDR1 were identified (Table I), which is ~2% of the genes sampled. Both increases and decreases in gene expression were observed. As shown in Table I, and in more detail in Table II, the selected genes can be divided into three different clusters. These are: (*a*) genes specifically affected by a particular peptide-oligonucleotide conjugate (genes affected by Ant 20 or by Ant 20 mismatch); (*b*) genes affected by treatment with both antisense and mismatch control conjugates (genes affected by both Ant 20 and Ant 20 mismatch); (*c*) genes affected by the presence of the Ant peptide (genes affected by Ant 20, Ant 20 mismatch, and Ant). Thus cells can respond through changes in gene expression to particular oligonucleotide sequences, to the intracellular presence of peptide-oligonucleotide conjugates in a sequence-indepen-

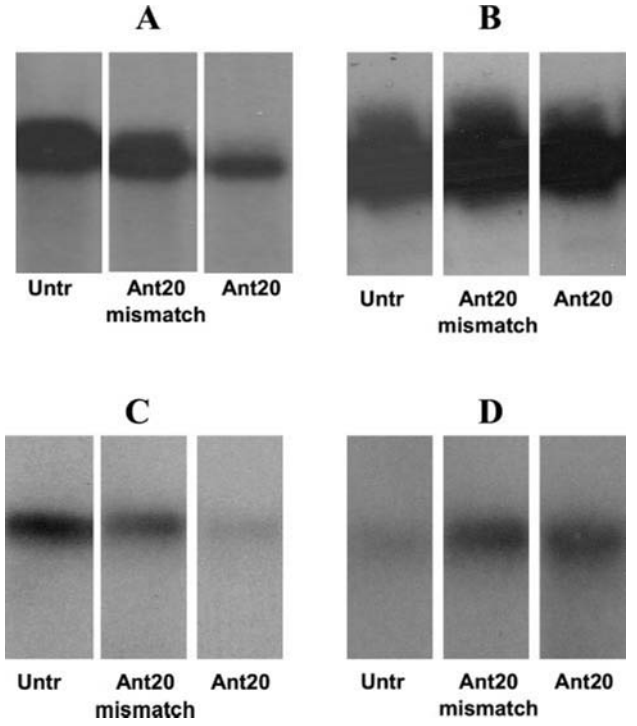

FIG. 3. **Northern blotting analysis.** Northern blotting was performed as described in the text. *A*, MDR1 gene; *B*, β-actin gene; *C*, human leukocyte tyrosine kinase gene; *D*, human PKCα gene. *Untr*, untreated control MES-SA/Dx5 cells; *Ant 20*, cells treated with antisense peptide-oligonucleotide conjugate; *Ant 20 mismatch*, cells treated with mismatch peptide-oligonucleotide conjugate.

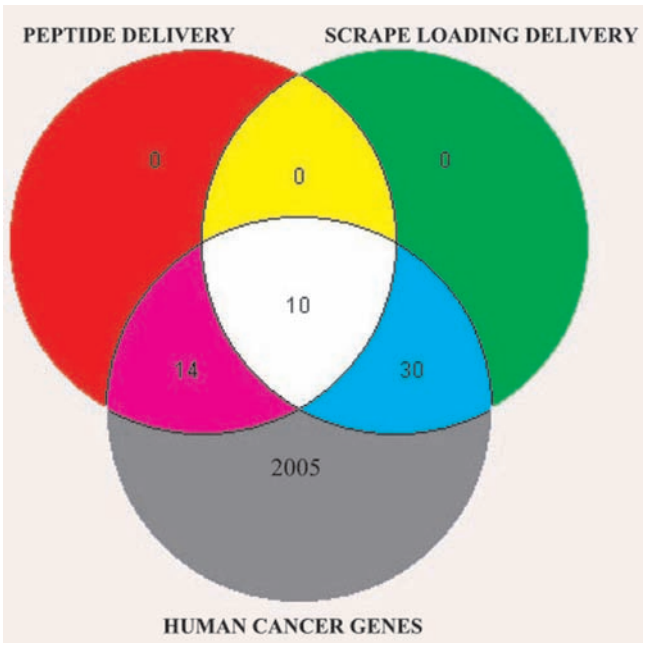

FIG. 4. **Venn diagram of genes affected by peptide-oligonucleotide conjugates *versus* free oligonucleotide.** The *diagram* compares the overlap of genes affected by anti-MDR1 peptide-oligonucleotide conjugates with those affected by free oligonucleotide delivered by scrape loading. The *white area* indicates the 10 genes affected by both treatments. The *purple area* indicates the 14 genes affected by the conjugates only (also described in Table II). The *blue-green area* indicates the 30 genes affected by scrape-loaded oligonucleotide only (the characteristics of these genes were not pursued further). The *gray area* represents the residual 2005 genes not affected by either treatment.

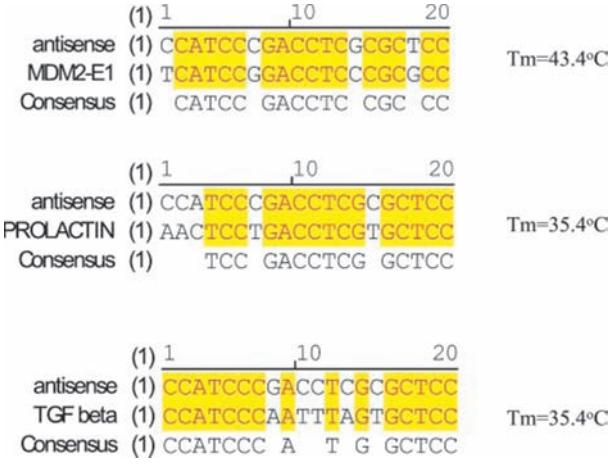

FIG. 5. **Sequence comparison of antisense oligonucleotides with down-regulated genes.** The three genes whose message levels were reduced by both anti-MDR1 peptide-oligonucleotide conjugate and by free anti-MDR1 oligonucleotide are indicated duplexed with the 20-mer anti-MDR1 oligonucleotide. Consensus sequences, mismatches, and predicted melting temperatures (*Tm*) are shown. *TGF*, transforming growth factor.

dent fashion, or to the presence of the polycationic delivery peptide. The affected genes did not belong to any obvious functional group or pathway.

These experiments were repeated in triplicate using a 10-fold lower concentration of anti-MDR1 peptide-oligonucleotide conjugate. As expected based on previous studies (12), there was a much weaker impact on P-glycoprotein levels as measured by flow cytometry and on MDR1 message levels measured by DNA array analysis (data not shown). Likewise there were fewer substantial changes in message levels of non-target genes with only 6 of the 24 genes previously identified as responsive to peptide-oligonucleotide conjugates showing changes that fit our criteria. Thus, there seem to be clear-cut dose-response relationships in this system for both the target gene and non-target genes.

DNA array technology gives the opportunity for simultaneous analysis of thousands of genes. However, because of the global character of this type of analysis and the possibility of errors, array data need to be confirmed by other independent approaches. To provide verification of the changes in message levels seen in the array analysis we performed Northern blots. Four different genes were chosen including MDR1, the target gene, β-actin as a "housekeeping gene" control, and two of the genes that responded significantly to treatment with peptide-oligonucleotide conjugates. According to the array data, leukocyte tyrosine kinase expression was predicted to be decreased by exposure to Ant 20 but not other treatments, whereas protein kinase C α was predicted to be increased by exposure to either Ant 20 or Ant 20 mismatch. As seen in Fig. 3, *A–D*, the Northern blot data confirmed the array analysis. Thus MDR1 and leukocyte tyrosine kinase message levels were decreased by exposure to Ant 20, whereas PKCα was increased by treatment with either conjugate. Actin message levels remained approximately constant. The magnitude of the decrease in MDR1 mRNA detected by Northern blotting, as well as the change in the level of P-glycoprotein (Fig. 1), were both about 4–5-fold, although the DNA array results (Fig. 2) indicated an average MDR1 message reduction of 3-fold. This seems a reasonable level of agreement given the very divergent assays used.

The experiments reported above have dealt with peptide-oligonucleotide conjugates or "free" peptide. However, one might ask whether the observed effects might be different if free antisense oligonucleotide was used instead. To address this issue, a 20-mer anti-MDR1 oligonucleotide was delivered

to cells using a "scrape-loading" process (16). This resulted in effective reduction in P-glycoprotein levels, similar to that attained with the anti-MDR1 peptide-oligonucleotide conjugate (evaluated by flow cytometry, data not shown). The same criteria were then used in these experiments, as in the experiments with peptide-oligonucleotide conjugates, to identify additional genes that we deemed to display significant changes in mRNA levels (Fig. 4). Interestingly, of the 24 genes whose mRNA levels were affected by the anti-MDR1 conjugate (see Table II), 10 were also affected by free antisense oligonucleotide delivered by scrape loading. The effects on these 10 genes likely represent actions of the antisense oligonucleotide moiety itself; conversely, effects on the other 14 genes probably are due to joint actions of the oligonucleotide and its associated delivery peptide. Thus, our observations indicate that there can be distinct effects on non-target genes because of the oligonucleotide alone, the delivery peptide alone, or the peptide-oligonucleotide conjugate.

In an attempt to better understand the basis of the observed changes in message levels upon treatment, the non-target genes were analyzed for possible direct interactions with the oligonucleotides used in this study. The complete sequences of all the genes that showed substantial changes in expression were recovered from the NCBI Draft Human Genome data base. The Vector NTI program was used in the Analyze Oligo Duplexes mode to compare possible expressed sequences with the sequence of the MDR1 antisense or mismatched oligonucleotides. Although no precise complementarities were observed, three of the genes whose message levels were reduced by both anti-MDR1 peptide-oligonucleotide conjugate and by scrape loading free anti-MDR1 oligonucleotide showed 75–80% complementarity, with predicted melting temperatures at or above physiological levels (Fig. 5). Thus these messages could potentially be targets for true antisense action. However, the lack of complementarity in many of the affected genes suggests that most of the observed changes in message levels in non-target genes were because of indirect effects of the oligonucleotides on cellular regulatory processes rather than to sequence-specific complexation with the mRNAs. Such indirect effects might include interactions with certain proteins in particular signaling and/or transcriptional regulation pathways.

Current results indicate that treatment of cells with peptide-antisense oligonucleotide conjugates can cause both specific reduction in the target mRNA as well as increases or decreases in a number of irrelevant mRNAs. These results buttress the concept that antisense experiments must be interpreted cautiously. However, our results do not negate the value of antisense as an experimental tool or a possible therapeutic approach. For several reasons, the results presented here may reflect an unusually high degree of nonspecific effects. First, because the MDR1 gene is a challenging target for antisense inhibition (20), we used rather high (μM) levels of oligonucleotide conjugates. In some other cases effects of comparable magnitude on the target gene have been obtained with much lower (nM) levels of antisense oligonucleotide (21); this would tend to reduce nonspecific interactions. Second, current studies involved phosphorothioate oligonucleotides; these are known to have substantially more nonspecific binding to proteins (22) than newer chemical forms of oligonucleotides (23) and thus a greater propensity for nonspecific effects (24). Finally, one should consider that, even in the present case, only 2% of the tested genes showed changes in message levels that were deemed to be significant according to our criteria, whereas most genes were not significantly affected. Thus it seems that antisense oligonucleotides can have substantial, though not perfect, selectivity as reagents for gene regulation.

It is interesting to note that the observations presented here provide quite a different picture than that resulting from another recent study of antisense effects using DNA array analysis (25). In that study antisense oligonucleotides were targeted to the message for one of the subunits of protein kinase A, a key growth regulatory protein. In addition to changes in PKA RIα subunit expression, many other changes in gene expression were observed as well as changes in cell growth; however, these were interpreted as being "downstream" of the effects on PKA. In our studies the levels of peptide-oligonucleotide conjugates used have no effect on cell growth (26), and thus the effects we see on non-target genes likely represent nonspecific actions of the antisense molecules rather than action on a coordinated growth regulatory program.

In summary, our results indicate that peptide-oligonucleotide conjugates, as well as unconjugated oligonucleotides, can have both specific antisense effects on target genes as well as significant nonspecific effects on irrelevant genes. These results do not contravene the utility of antisense as a research tool or treatment modality. However, proper interpretation of antisense studies should include the best tools available for evaluating selectivity; clearly DNA arrays will be important in that regard.

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