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Review paper

Application of peptide nucleic acid in cancer therapy

Helle Knudsen and Peter E Nielsen
Center for Biomolecular Recognition, Department of Medical Biochemistry & Genetics, Biochemistry Laboratory B, The Panum Institute, Blegdamsvej 3c, 2200 Copenhagen N, Denmark.
Tel: (+45) 35327762; Fax: (+45) 31396042.

Peptide nucleic acid (PNA) is a DNA mimic with a pseudo-peptide backbone composed of aminoethyl glycine units. Several features of PNA, such as superior hybridization affinities to RNA and DNA, high biological stability, and convenient solid-phase synthesis, make it a promising candidate for use as a gene targeting drug. Here we review the chemical and biological properties of PNA in relation to its potential as an anti-cancer drug.

Key words: Anti-gene, antisense, cancer therapy, PNA.

Introduction

The discovery of oncogenes and tumor supressor genes, and their role in transformation of normal cells into malignant cells has opened new areas of research in cancer therapy. Selective inhibition of gene expression by using DNA or RNA binding agents is an attractive approach to modulate the activity of such genes. Oligonucleotides, short synthetic fragments of nucleic acids, can be designed to recognize and hybridize to complementary sequences in a particular mRNA and thereby inhibit its translation (the antisense strategy). Alternatively, oligonucleotides can be targeted to double-stranded (ds) DNA and thus interfere with transcription of a particular gene (the anti-gene strategy). These two approaches are depicted in Figure 1.

Oligonucleotides containing natural phosphodiester linkages are highly susceptible to degradation by cellular nucleases. Consequently, a large number of nuclease-resistant DNA analogs and mimics has been developed. Although most of these compounds have improved properties, problems concerning non-specific effects and cellular uptake still exist.[1–6]

Peptide nucleic acid (PNA) is a nucleic acid mimic showing promising potential as gene-specific drug.

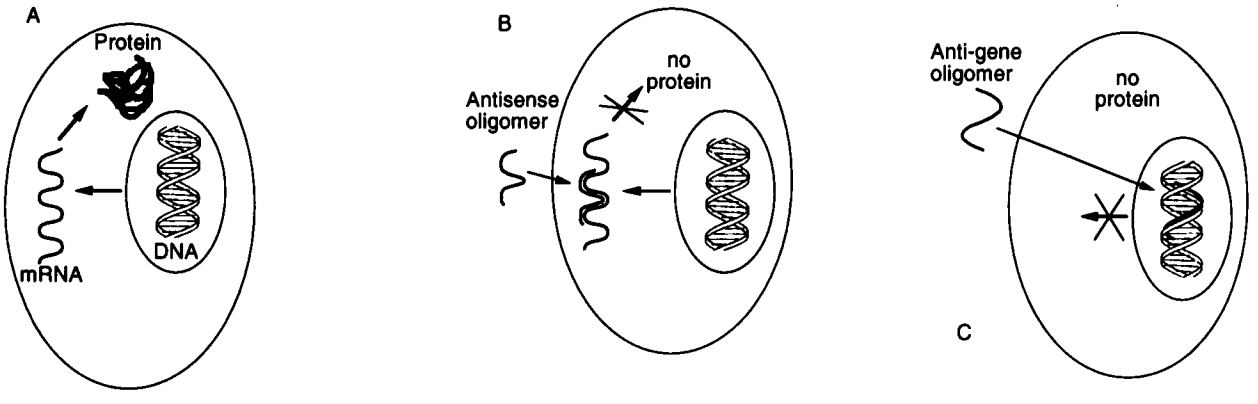

Figure 1. A schematic representation of the principles in the antisense and anti-gene strategies. In untreated cells (A), DNA is transcribed into mRNA, which is then translated into protein. When the cells are treated with an antisense oligomer hybridizing to a sequence in a specific mRNA, expression of the protein is inhibited at the level of translation (B). Treatment of the cells with an anti-gene oligomer complementary to a sequence in the DNA leads to inhibition of transcription of the gene into mRNA (C).

Correspondence to PE Nielsen

PNA has a pseudo-peptide backbone composed of charge neutral and achiral *N*-(2-aminoethyl)glycine units to which the nucleobases are attached via methylene carbonyl linkers (Figure 2).[7–11] PNA hybridizes with high affinity to complementary sequences in single-stranded (ss) DNA or RNA forming Watson–Crick double helices.[7,12,13] Notably, homopyrimidine PNA binds to complementary sequences in dsDNA by a strand displacement mechanism. This involves Watson–Crick binding of one PNA and Hoogsteen binding of another PNA to the complementary purine DNA strand, whereas the non-complementary DNA strand is displaced as a loop (Figure 3). Such complexes are extremely stable.[15,16] Likewise, homopyrimidine PNAs form thermally extremely stable PNA_2–RNA triplexes with targets in RNA.[9,10,14] In spite of the high thermal stability of PNA–RNA and PNA–DNA complexes, the binding is highly sensitive to mismatches,[7,17,18] an important property with respect to specificity. Employing conventional peptide chemistry it is possible to synthesize PNA in milligram to gram quantities.[19] For an exhaustive description of the chemistry and structural features of PNA the reader is refered to reviews by Hyrup and Nielsen[20] and Eriksson and Nielsen,[21] respectively.

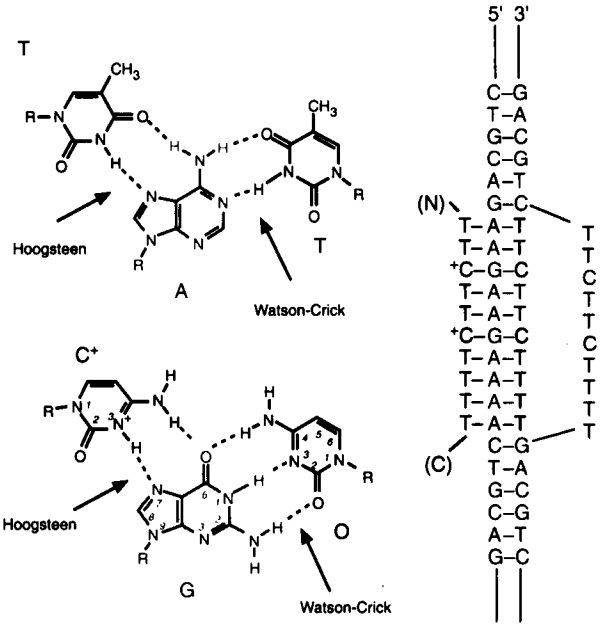

Figure 3. Chemical structures of T*A–T and C^+*G–C base triplets, which are responsible for PNA_2–DNA triplex formation. To the right a schematic drawing of a PNA–dsDNA strand displacement complex. The most stable complex is formed with the Watson–Crick PNA strand being antiparallel and the Hoogsteen PNA strand being parallel to the DNA target strand.

Antisense properties

The cellular endonuclease ribonuclease H (RNase H) cleaves the RNA moity of DNA–RNA heteroduplexes and is believed to play a crusial role in antisense effects observed for phosphodiester and phosphorothioate oligonucleotides.[22,23] However, complexes of RNA and oligonucleotides with modified backbones are usually not recognized by RNase H (the phosphorothioates excepted) and this is also the case for PNA–RNA duplexes.[24] Therefore, PNA-mediated antisense effects will be dependent on mechanisms involving sterical blocking of either RNA processing, transport into cytoplasm or translation.

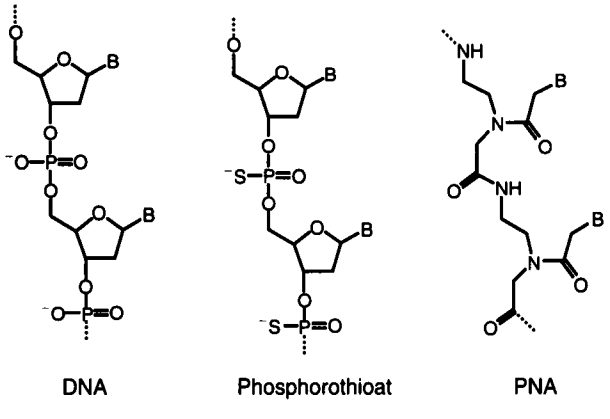

Figure 2. The chemical structures of regular DNA, phosphorothioate DNA and PNA, respectively.

In theory, it should be possible to combine the high stability of PNA–RNA complexes with the RNase H activating potential of DNA–RNA complexes by making PNA–DNA chimeras. Such compounds have indeed been synthesized, but their hybridization properties are so far not optimal and no antisense effects have been reported.[25–28]

PNA does not readily enter cells in culture (see below) and reports on antisense activity of PNA therefore mainly involve *in vitro* studies. Initially, Hanvey *et al.*[29] demonstrated that a 15-mer pyrimidine-rich PNA (presumably triplex forming) complementary to a sequence in the interleukin-2 receptor α (IL-2Rα) subunit mRNA was able to inhibit its translation in rabbit reticulocyte lysates. Recently, we reported a more detailed investigation of PNAs antisense properties *in vitro*.[24] Using translation of chloramphenicol acetyltransferase (CAT) mRNA in rabbit reticulocyte lysates as a model system, we found that a 15-mer PNA with mixed purine/pyrimidine sequence (duplex forming) blocked

translation initiation of CAT specifically when targeted towards a sequence overlapping the AUG start codon. Duplex forming PNAs hybridizing to sequences in the coding region of the CAT mRNA, however, did not inhibit translation elongation. Apparently, the PNA–RNA duplex is not sufficiently stable to arrest the ribosome. In contrast, triplex forming PNAs targeted towards the same region did block translation efficiently. Even a 6-mer bis-PNA (in which the two hybridizing PNA strands are linked[34]) showed specific inhibition. Presumably, the more bulky complexes formed with the homopyrimidine PNAs work as an efficient 'road-block' capable of disturbing the translation machinery.

Similar results have been obtained using a more cancer-related system. In this case PNAs were designed to hybridize to sequences in the promyelocytic leukemia/retinoic acid receptor α (PML/RARα) fusion gene present in acute promyelocytic leukemia (APL).[30] A duplex forming PNA complementary to a sequence in the AUG start region as well as a triplex forming PNA targeted towards a poly-purine sequence in the coding region of PML/RARα mRNA efficiently blocked translation, whereas translation was unaffected when a duplex forming PNA was hybridized to a target in the coding region of PML/RARα mRNA.

The antisense properties of PNA in a cellular context was assessed by microinjecting PNA directly into the nuclei of cultured cells, thus bypassing the poor uptake rate.[29,31] A 15-mer homopyrimidine PNA complementary to a sequence cloned into the 5′ untranslated region (5′ UTR) of SV40 T antigen RNA was able to inhibit translation of T antigen specifically. When the same PNA was targeted at complementary sequences in the coding region of SV40 T Ag mRNA translation inhibition again was obtained; however, not as efficiently as with the 5′ UTR target.[31]

In conclusion, studies performed in *in vitro* systems indicate that PNA is a potent antisense mediator. From the results obtained so far it can be concluded that translation initiation can be inhibited by using PNAs with mixed purine/pyrimidine sequences, forming duplexes with their target, whereas inhibition of translation elongation requires triplex forming homopyrimidine PNAs.

Anti-gene properties

When targeting genomic DNA by the anti-gene strategy, inhibition can in theory be obtained by formation of just one PNA–DNA complex in each haploid genome. In contrast, when the target is mRNA, PNA must form complexes with the large number of mRNA present in the cell in order to inhibit translation. In this respect the anti-gene strategy may be favored.

Since homo-pyrimidine PNAs form extremely stable strand displacement complexes upon binding to dsDNA, they should be excellent anti-gene agents. It has been shown that such PNA_2–dsDNA complexes are indeed able to arrest elongation of both prokaryotic and eukaryotic RNA polymerases *in vitro*.[29,32]

In terms of therapeutic application, several issues are, however, unclear. First, in order to make PNA bind dsDNA, a poly-purine target must be present in the gene of interest. This of course limits the number of possible targets within a gene. Second, the formation of strand displacement complexes occurs very slowly at physiological salt concentrations, despite the fact that the complexes, once formed, are stable at high ionic strength. However, improvements of the binding rate have been obtained by constructing positively charged bis-PNAs, in which the 'Watson–Crick' and 'Hoogsteen' strands are chemically linked. Especially, coupling lysine residues to the PNA increases their association rate with dsDNA.[33] In addition, the binding of the 'Hoogsteen PNA strand' has been made pH independent by incorporation of pseudoisocytosine instead of cytosine in the 'Hoogsteen' strand.[34] It must be taken into account that binding of PNA to double-stranded targets could be different in an *in vivo* context compared to the *in vitro* situation. It has been found that binding of PNA to supercoiled plasmid DNA was much faster compared to relaxed or linear DNA when assayed at physiological salt concentrations,[35] indicating that the topology of DNA is an important parameter for the binding rate. Since transcriptionally active chromosomal DNA usually is negatively supercoiled,[36,37] this could be of relevance for PNA binding *in vivo*. Furthermore, it has been shown that binding of PNA to dsDNA is greatly enhanced when the DNA is being transcribed, probably due to the presence of partly ssDNA in the transcription bubble.[38]

Two studies have addressed whether PNA targeted to gene regulatory regions could modulate transcription. Vickers *et al.*[39] showed that binding of a PNA 15-mer to a sequence overlapping a transcription factor NF-κB site in the IL2-Rα promoter specifically inhibited NF-κB binding and activation of transcription both in eukaryotic nuclear extracts and in cultured cells transfected with an IL2-Rα reporter plasmid containing the bound PNA. Praseuth *et al.*

performed similar experiments, but found that a PNA 10-mer failed to inhibit transcriptional activation inside cells.[40] The discrepancy of the two studies could be due to use of different PNAs, different DNA constructs and/or different transfection protocols.

It has been reported that a PNA 18-mer containing both purines and pyrimidines showed anti-gene activity in the nuclei of lysolecithin-permeabilized cells.[41] It should be emphasized, however, that strand invasion has not been observed *in vitro* with mixed purine/pyrimidine PNAs. It is therefore not fully clear by which mechanism this inhibition occurs.

In conclusion, there seems to be potential for developing PNA into an anti-gene agent. However, more studies involving both *in vitro* and *in vivo* systems will be needed in order to investigate fully the anti-gene properties of PNA.

Inhibition of telomerase activity

Recently, Norton *et al.*[42] demonstrated a new potential PNA target, using PNA to inhibit human telomerase activity. Telomerase is the enzyme responsible for maintaining the length of telomers (chromosome ends) during replication. In contrast to normal somatic cells, the majority of cancer cells has been found to have telomerase activity and telomerase has therefore been proposed to be a potential target for cancer therapy.

Telomerase carries an RNA which function as a template for extension of the chromosome ends. PNAs complementary to a sequence in this RNA template were shown to work as very efficient and sequence-specific inhibitors of telomerase activity both *in vitro* and in permeabilized cells. A comparison of the ability of PNA and phosphorothioate oligonucleotides, respectively, to inhibit telomerase activity showed that PNA possed clear advantages: lower concentrations of PNA were needed for specific inhibition, whereas the phosphorothioate oligonucleotides worked in a non-sequence-specific way.[42]

Delivery of PNA

The prospect of using PNA as a gene therapeutic drug makes sense only if PNA is delivered efficiently to cells and distributed in a way that will enable interaction with the target mRNA or DNA. Studies conducted with cells in culture indicate that PNA uptake is slow and that PNA seems to end up in endosomal compartments.[29,43] These observations would predict rather poor bioavailability of PNA *in vivo*. However, phosphorothioate oligonucleotides, which have also shown inefficient uptake in cell culture, seem to have a favorable pharmacokinetic behavior *in vivo* and have been shown to be effective in various animal models.[5] It is therefore essential that the *in vitro* studies of PNA uptake are extended with studies of uptake in whole animals in order to get a more realistic picture of PNA behavior. Anyway, it is possible that cellular uptake of PNA could be facilitated by conjugation to carrier molecules or incorporation into liposomes, as has been observed for oligonucleotides.

In agreement with its chemical remoteness to natural nucleic acids or peptides, PNA exhibits resistance to degradation by cellular nucleases and proteinases, and remains intact when incubated in the presence of serum or cellular extracts.[44] Furthermore, there have not been any signs of general toxicity by PNA. These facts are of course other important features with respect to therapeutic uses of PNA.

Conclusion and perspectives

The extensive work done with phosphorothioates and other nucleic acid analogs has provided usefull guidelines in the search for suitable targets in antisense and anti-gene based cancer therapy. A large number of genes suspected to play important roles in the development of tumors have been investigated (Table 1). Anti-tumor activity has been observed in many of these experiments, but the results seem to be much dependent on the assay system employed. Furthermore, some of the observed effects using oligonucleotides have turned out to

Table 1. Some examples of cancer related genes targeted by antisense oligonucleotides

Target	Model	Reference
c-*myc*	human melanoma cells/*in vivo* (mice)	45
	in vivo (mice)	46
	human glioma cells	47
	smooth muscle cells	48
c-*myb*	smooth muscle cells	48
	human myeloid leukemia cells	49
	in vivo (mice)	50
Ha-*ras*	*in vivo* (mice)	51
	in vivo (mice)	52
bcl-2	human lymphoma cells	53
	human lymphoma cells	54
bcr-abl	myeloid leukemia cells	55
	leukemic cells	56

take place by non-specific or non-antisense mechanisms. Nonetheless, recent results using the second generation of modified oligonucleotides both in cell culture and in mice are very promising,[2,57] and phosphorothioates are now in clinical trials.[1]

With respect to developing PNA into a therapeutic anti-cancer drug there is still some way to go. In particular, data on PNA's behavior *in vivo* would be essential. In addition, studies of PNAs antisense and anti-gene properties in cell culture systems may provide useful directions for future developments. The *in vitro* studies performed so far have, nevertheless, suggested that PNA has the potential of being an efficient and specific gene targeted drug with some clear advantages over conventional phosphodiester and phosphorothioate oligonucleotides.

The chemistry of the PNA backbone allows for a wide variety of modifications, thus opening possibilities of constructing multi-functional backbones that could be used for optimizing, for example, the binding properties, bioavailability and pharmacokinetics of PNA. Furthermore, development of modified bases that will enable PNA to strand invade and/or form triplexes with mixed purine/pyrimidine targets is an active field of research. Only the future will reveal if PNA can hold its promise as a therapeutic lead compound.

References

1. Agrawal S. Antisense oligonucleotides: towards clinical trials. *Trends Biotechnol* 1996; **14**: 376–87.
2. Altmann KH, Dean NM, Fabbro D, *et al.* Second generation of antisense oligonucleotides: from nuclease resistance to biological efficacy in animals. *Chimia* 1996; **50**: 168–76.
3. Tidd DM. Specificity of antisense oligonucleotides. *Perspect Drug Discovery Design* 1996; **4**: 51–60.
4. Agrawal S, Iyer RP. Modified oligonucleotides as therapeutic and diagnostic agents. *Curr Biol* 1995; **6**: 12–9.
5. Sharma HW, Narayanan R. The therapeutic potential of antisense oligonucleotides. *BioAssays* 1995; **17**: 1055–63.
6. Stein CA, Narayanan R. Antisense oligodeoxynucleotides. *Curr Opin Oncol* 1994; **6**: 587–94.
7. Egholm M, Buchardt O, Christensen L, *et al.* PNA hybridizes to complementary oligonucleotides obeying the Watson–Crick hydrogen-bonding rules. *Nature* 1993; **365**: 566–8.
8. Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* 1991; **254**: 1497–500.
9. Egholm M, Buchardt O, Nielsen PE, Berg RH. Peptide nucleic acids (PNA). Oligonucleotide analogues with an achiral peptide backbone. *J Am Chem Soc* 1992; **114**: 1895–7.
10. Egholm M, Nielsen PE, Buchardt O, Berg RH. Recognition of guanine and adenine in DNA by cytosine and thymine containing peptide nucleic acids (PNA). *J Am Chem Soc* 1992; **114**: 9677–8.
11. Nielsen PE, Egholm M, Buchardt O. Peptide nucleic acid (PNA). A DNA mimic with a peptide backbone. *Bioconjugate Chem* 1994; **5**: 3–7
12. Leijon M, Graslund A, Nielsen PE, *et al.* Structural characterization of PNA–DNA duplexes by NMR. Evidence for DNA in a B-like conformation. *Biochemistry* 1994; **33**: 9820–5.
13. Brown SC, Thomson SA, Veal JM, Davis DG. NMR solution structure of a peptide nucleic acid complexed with RNA. *Science* 1994; **265**: 777–80.
14. Kim SK, Nielsen PE, Egholm M, Buchardt O, Berg RH, Norden B. Right-handed triplex formed between peptide nucleic acid PNA-T_8 and poly(dA) shown by linear and circular dichroism spectroscopy. *J Am Chem Soc* 1993; **115**: 6477–81.
15. Nielsen PE, Egholm M, Buchardt O. Evidence for $(PNA)_2$/DNA triplex structure upon binding of PNA to dsDNA by strand displacement. *J Mol Recognit* 1994; **7**: 165–70.
16. Cherny DY, Belotserkovskii BP, Frank-Kamenetskii MD, *et al.* DNA unwinding upon strand-displacement binding of a thymine-substituted polyamide to double-stranded DNA. *Proc Natl Acad Sci USA* 1993; **90**: 1667–70.
17. Nielsen PE, Egholm M, Berg RH, Buchardt O. Peptide nucleic acids (PNAs): potential antisense and anti-gene agents. *Anti-Cancer Drug Design* 1993; **8**: 53–63.
18. Jensen KK, Ørum H, Nielsen PE, Norden B. Hybridization kinetics of peptide nucleic acids (PNA) with DNA and RNA studied with BIAcore technique. *Submitted.*
19. Christensen L, Fitzpatrick R, Gildea B, *et al.* Solid-phase synthesis of peptide nucleic acids. *J Peptide Sci* 1995; **3**: 175–83.
20. Hyrup B, Nielsen PE. Peptide nucleic acids (PNA): synthesis, properties and potential applications. *Bioorg Biomed Chem* 1996; **4**: 5–23.
21. Eriksson M, Nielsen PE. PNA–nucleic acid complexes. Structure, stability and dynamics. *Quart Rev Biophys* 1996; in press.
22. Giles RV, Ruddell CJ, Spiller DG, Green JA, Tidd DM. Single base discrimination for ribonuclease H-dependent antisense effects within intact human leukaemia cells. *Nucleic Acids Res* 1995; **23**: 954–61.
23. Walder RY, Walder JA. Role of RNase H in hybrid-arrested translation by antisense oligonucleotides. *Proc Natl Acad Sci USA* 1988; **85**: 5011–5.
24. Knudsen H, Nielsen PE. Antisense properties of duplix- and triplex-forming PNAs. *Nucleic Acids Res* 1996; **24**: 494–500.
25. Stetsenko DA, Lubyako EN, Potapov VK, Azhikina TL, Sverdlov ED. New approach to solid phase synthesis of polyamide nucleic acid analogues (PNA) and PNA–DNA conjugates. *Tetrahedron Lett* 1996; **37**: 3571–4.
26. Finn PJ, Gibson NJ, Fallon P, Hamilton A, Brown T. Synthesis and properties of DNA–PNA chimeric oligomers. *Nucleic Acids Res* 1996; **24**: 3357–64.
27. van der Laan AC, Meeuwenoord NJ, Kuyl-Yeheskiely E, Oosting RS, Brands R, van Boom JH. Solid support synthesis of a PNA–DNA hybrid. *Recueil des Tra-*

veaux Chimiques des Pays-Bas 1995; **114**: 295–7.
28. Bergmann F, Bannwarth W, Tam S. Solid phase synthesis of directly linked PNA–DNA hybrids. *Tetrahedron Lett* 1995; **36**: 6823–6.
29. Hanvey JC, Peffer NJ, Bisi JE, *et al.* Antisense and antigene properties of peptide nucleic acids. *Science* 1992; **258**: 1481–5.
30. Gambacorti-Passerini C, Mologni L, Bertazzoli C, *et al.* *In vitro* transcription and translation inhibition by anti-Promyelocytic Leukemia (PML)/Retinoic acid receptor α and anti-PML peptide nucleic acid. *Blood* 1996; **88**: 1411–7.
31. Bonham MA, Brown S, Boyd AL, *et al.* An assessment of the antisense properties of RNase H-competent and steric-blocking oligomers. *Nucleic Acids Res* 1995; **23**: 1197–203.
32. Nielsen PE, Egholm M, Buchardt O. Sequence-specific transcription arrest by nucleic acid bound to the DNA template strand. *Gene* 1994; **149**: 139–45.
33. Demidov VV, Nielsen PE. Unpublished data.
34. Egholm M, Christensen L, Dueholm KL, Buchardt O, Coull J, Nielsen PE. Efficient pH-independent sequence-specific DNA binding by pseudoisocytosine-containing bis-PNA. *Nucleic Acids Res* 1995; **23**: 217–22.
35. Bentin T, Nielsen PE. Enhanced peptide nucleic acid binding to supercoiled DNA: possible implications for 'breathing' dynamics. *Biochemistry* 1996; **35**: 8863–9.
36. Ljungman M, Hanawalt PC. Presence of negative torsional tension in the promoter region of transcriptionally poised dihydropholate reductase gene *in vivo*. *Nucleic Acids Res* 1995; **23**: 1782–9.
37. Jupe ER, Sinden RR, Cartwright IL. Stably maintained microdomain of localized unrestrained supercoiling at a *Drosophila* heat shock gene locus. *EMBO J.* 1993; **12**: 1067–75.
38. Larsen HJ, Nielsen PE. Transcription-mediated binding of peptide nucleic acid (PNA) to double stranded DNA: sequence-specific suicide transcription. *Nucleic Acids Res* 1996; **24**: 458–63.
39. Vickers TA, Griffith MC, Ramasamy K, Risen LM, Freier SM. Inhibition of NF-κB specific transcriptional activation by PNA strand invation. *Nucleic Acids Res* 1995; **23**: 3003–8.
40. Praseuth D, Grigoriev M, Guieysse A, *et al.* Peptide nucleic acids directed to the promotor of the α-chain of the promotor of the interleukin 2 receptor. *Biochem Biophys Acta* 1996; **1309**: 226–38
41. Boffa LC, Morris PL, Carpaneto EM, Louissaint M, Allfrey VG. Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence. *J Biol Chem* 1996; **271**: 13228–33.
42. Norton JC, Piatyszek MA, Wright WE, Shay JW, Corey DR. Inhibition of human telomerase activity by peptide nucleic acids. *Nature Biotechnol* 1996; **14**: 615–9.
43. Knudsen H and Nielsen PE. Unpublished data.
44. Demidov VV, Potaman VN, Frank-Kamenetskii MD, *et al.* Stability of peptide nucleic acids in human serum and cellular extracts. *Biochem Pharmacol* 1994; **48**: 1310–3.
45. Leonetti C, D'Agnano I, Lozupone F, *et al.* Antitumor effect of c-*myc* antisense phosphorothioate oligodeoxynucleotides on human melanoma cells *in vitro* and in mice. *J Natl Cancer Inst* 1996; **88**: 419–29.
46. Huang Y, Snyder R, Kligshteyn M, Wickstrom E. Prevention of tumor formation in a mouse modes of Burkitt's lymphoma by 6 weeks of treatment with anti-c-*myc* DNA phosphorothioate. *Mol Med* 1995; **1**: 647–58.
47. Chavany C, Connell Y, Neckers L. Contribition of sequence and phosphorothioate content to inhibition of cell growth and adhesion caused by c-*myc* antisense oligomers. *Mol Pharmacol* 1995; **48**: 738–46.
48. Burgess TL, Fisher EF, Ross SL, *et al.* The antiproliferative activity of c-*myb* and c-*myc* antisense oligonucleotides in smooth muscle cells is caused by a nonantisense mechanism. *Proc Natl Acad Sci USA* 1995; **92**: 4051–5.
49. Citro G, Szczylik C, Ginobbi P, Zupi G, Calabretta B. Inhibition of leukaemia cell proliferation by folic acid-polylysine-mediated introduction of c-*myc* antisense oligodeoxynucleotides into HL-60 cells. *Br J Cancer* 1994; **69**: 463–7.
50. Ratajczak MZ, Kant JA, Luger SM, *et al.* *In vivo* treatment of human leukemia in a scid mouse model with c-*myb* antisense oligodeoxynucleotides. *Proc Natl Acad Sci USA* 1992; **89**: 11823–7.
51. Schwab G, Chavany C, Doroux I, *et al.* Antisense oligonucleotides absorbed to polyalkylcyanoacrylate nonoparticles specifically inhibit mutated Ha-*ras*-mediated cell proliferation and tumorgenicity in nude mice. *Proc Natl Acad Sci USA* 1994; **91**: 10460–4.
52. Gray GD, Hernandez OM, Hebel D, Root M, Pow-Sang JM, Wickstrom E. Antisense DNA inhibition of tumor growth by c-Ha-*ras* oncogene in nude mice. *Cancer Res* 1993; **53**: 577–80.
53. Smith MR, Abubakr Y, Mohammad R, Xie T, Hamdan M, al-Katib A. Antisense oligodeoxyribonucleotide down-regulation of *bcl-2* gene expression inhibits growth of the low-grade non-Hodgkin's lymphoma cell line WSU-FSCCL. *Cancer Gene Therapy* 1995; **2**: 207–12.
54. Kitada S, Takayama S, De Riel K, Tanaka S, Reed JC. Reversal of chemoresistance of lymphoma cells by antisense-mediated reduction of *bcl-2* gene expression. *Antisense Res Dev* 1994; **4**: 71–9.
55. Vaerman JL, Lammineur C, Moureau P, *et al.* BCR-ABL antisense oligodeoxyribonucleotides suppress the growth of leukemic and normal hematopoietic cells by a sequence-specific but nonantisense mechanism. *Blood* 1995; **86**: 3891–6.
56. Chasty R, Whetton A, Lucas G. A comparison of the effect of *bcr/abl* breakpoint specific phosphorothioate oligodeoxynucleotides on colony formation by *bcr/abl* positive and negative, CD34 enriched mononuclear cell populations. *Leukemia Res* 1996; **20**: 391–5.
57. Monia BP, Johnston JF, Geiger T, Muller M, Fabbro D. Antitumor activity of a phosphorothioate antisense oligonucleotide targeted against C-*raf* kinase. *Nature Medicine* 1996; **2**: 668–75.

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