Antisense Oligodeoxynucleotide Technology: Potential Use for the Treatment of Malignant Brain Tumors

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The use of antisense oligodeoxynucleotides as a possible treatment for patients with brain tumors is promising due to their specificity, ease of production, and lack of adverse effects.

Background: Antisense oligodeoxynucleotides (ODNs) have been proposed as a new therapy for patients with cancer, including malignant brain tumors. Antisense ODNs are taken up by tumor cells and selectively block gene expression. Use of ODNs for brain tumors is attractive due to their theoretical specificity, relative ease of production and, to date, paucity of reported adverse effects. This article presents current information regarding antisense ODNs and their possible future use for the treatment of brain tumors.

Methods: The available published experimental and clinical information regarding antisense ODN treatment of glioblastoma cells and administration into the central nervous system (CNS) was reviewed. Other clinically relevant information pertaining to the molecular biology of antisense ODNs was also collected and summarized.

Results: Targets for antisense ODN therapy in malignant glioma cells have included c-myc, c-myb, c-sis, c-erb B, CD44, p34cdc2, bFGF, PDGF, TGF-beta, IGF-1, PKC-alpha tumor necrosis factor, urokinase, and S100 beta protein. Few in vivo studies of ODN treatment of brain tumors have yet been reported. Systemically administered ODNs enter the brain only in extremely small quantities; therefore, microinfusion into the brain has been recommended.

Conclusions: Antisense ODNs have been used successfully to block glioblastoma gene expression in vitro and expression of multiple genes within the CNS of experimental animals. Upcoming clinical trials will address the safety of antisense ODN use against malignant brain tumors.

Introduction

The concept of antisense-mediated gene inhibition, first introduced by Stephenson and Zamecnik1 in 1978, has now emerged as a potentially powerful alternative or adjunct to conventional cancer chemotherapy.2-9 This is particularly exciting in the case of malignant astrocytomas, where results with traditional chemotherapy have been disappointing. The discovery that synthetic fragments of DNA can inhibit the transcription and/or translation of selected genes in a sequence-specific manner initially opened up a new mechanism for analyzing gene function, then launched a new field of drug development in which early clinical trials are now proceeding.4,10-13

Clinical applications for antisense ODNs have been envisioned in many fields including oncology, vascular and genetic diseases, and the treatment of the human immunodeficiency virus and other viral infections.7,12,14-19 The term "antisense" refers to the fact that the nucleic acid synthesized is complementary to the coding (ie, "sense") genetic sequence of the target gene.10 Antisense constructs hybridize in an antiparallel orientation to nascent mRNA through Watson-Crick base-pairing.12 Theoretically, an oligomer 17 nucleotides in length should find a unique target within the 3 x 109 base pairs of the human genome.2,12,20

To date, two main antisense strategies have been employed: transfection of cells with antisense cDNA and treatment of cells with the shorter antisense oligodeoxynucleotides (ODNs). The former strategy has been successfully used in vitro against glioblastoma cells with gene targets such as basic fibroblast growth factor (bFGF),21 or protein kinase C isotype a (PKCa),22 and in animal glioma models targeting insulin-like growth factor 1 (IGF-1)23 or vascular endothelial growth factor (VEGF).24,25 The latter strategy has been more widely used, however, and is the focus of this review. In comparison to the cDNA approach, antisense ODNs are easier to synthesize and obviate the need for a viral vector for delivery to cells.

In order to be useful therapeutically, an ODN must (1) exhibit reasonable stability in the physiologic (or pathologic!) environment, (2) be taken up and retained in adequate quantities by the target (here, neoplastic) cells, (3) specifically bind target mRNA with high affinity, (4) have an acceptable therapeutic ratio, being free of unwanted toxic and nonspecific side effects, and (5) be easily synthesized in sufficient quantities to allow clinical use.3,10,26,27 Most of these criteria have already been met by phosphorothioated ODNs (described below); yet, second-generation ODNs are already under development.

ODN Modifications and Cellular Uptake

Unmodified ODNs are polyanions with a phosphodiester backbone (Fig 1A). They are rapidly degraded under physiologic conditions by single-stranded nucleases, primarily 3'-exonucleases.3,26 Because of this, ODN modifications have been designed to retard degradation. The phosphorothioate modification of the oligonucleotide backbone (Fig 1B), in which a sulphur atom replaces one of the nonbridging oxygen atoms in the phosphate group,3 produces ODNs that are relatively resistant to cellular and serum nucleases. Phosphorothioated ODNs have been the type most commonly used in investigations to date, including studies of malignant glioma.8,10,11,28-35 Another variation of the backbone produces the methylphosphonate modification (Fig 1C).36,37 Methylphosphonate ODNs have no net charge,
which aids in preventing nuclease digestion but also decreases water solubility. In considering access to the CNS, however, the use of the more lipophilic methylphosphonates could be advantageous. The phosphoramidate modification (Fig 1D) has more recently been described. Phosphoramidates may offer advantages over phosphorothioated ODNs, such as decreased nonspecific binding to proteins.

Other modifications of ODN structure have been designed to increase cellular uptake. Enhanced delivery of ODNs to cells has been achieved by combining them with synthetic cationic lipids or by linking them to hydrophobic moieties such as porphyrin or cholesterol. Enhancement of receptor-mediated uptake has also been described, for example, by conjugating ODNs to a polysine complex with or without transferrin. The use of liposomes as delivery vehicles has been used to increase cellular uptake and protect from extracellular degradation.

Cellular uptake of ODNs occurs by means of fluid-phase pinocytosis and/or receptor-mediated endocytosis. Other modifications of ODN structure have been designed to increase cellular uptake. Enhanced delivery of ODNs to cells has been achieved by combining them with synthetic cationic lipids or by linking them to hydrophobic moieties such as porphyrin or cholesterol. Enhancement of receptor-mediated uptake has also been described, for example, by conjugating ODNs to a polysine complex with or without transferrin.

Mechanisms of Antisense ODN Action

Although the mechanisms of antisense ODN action continue to be elucidated, on a fundamental level, antisense ODNs bind the target mRNA template, thus blocking successful translation of the corresponding protein. High-affinity binding can occur in the cytoplasm (to mRNA) and/or nucleus (to hnRNA) after passage through nuclear pores. The formation of the DNA:RNA heteroduplex results in gene inactivation either through steric blocking of the ribosome complex or by triggering mRNA cleavage by RNase H. Cleavage is rapidly followed by further degradation and is therefore an irreversible process.

Antisense ODN constructs also can interfere with transcription by a process called triple-helix formation (the “antigene” strategy), in which the ODN binds to double-stranded DNA in the nucleus. Genetic targets for this type of strategy, however, are more limited since they must be pure polypurine tracts.

Theoretically, antisense ODNs could also act by (1) hybridizing to open DNA loops created by RNA polymerase or at intron-exon junctions, (2) interfering with mRNA splicing or transport of mRNA from the nucleus to the cytoplasm, or (3) interfering with translation through inhibition of the binding of initiation factors or assembly of ribosomal subunits at the start codon, among other possibilities. Antisense agents called “ribozymes” have been designed that induce catalytic cleavage of target RNA by the addition of a sequence that has natural self-splicing activity. In carrying out the cleavage, the ribozyme itself is not altered and thus is capable of continuing to cleave other molecules.

Targets for Antisense ODNs in Malignant Brain Tumor Cells

Given the continued poor prognosis of patients with malignant brain tumors, many investigators have suggested that ODN therapy might be useful. Reported target genes for antisense ODN therapy in malignant glioma cells (Table) have included bFGF, c-myc, c-sis, c-erb B, c-myb, CD44, p34cdc2, platelet-derived growth factor, transforming growth factor-beta, IGF-1, PKC-alpha, tumor necrosis factor, urokinase, and the S100beta protein. In the studies using these target genes, mRNA and/or protein expression was successfully down-regulated, often with corresponding decreases in tumor cell growth or other neoplastic phenotype. Usually, the more specific part of the mRNA that is targeted is at the 5’ end of the transcript, spanning the translation initiation codon. The number of additional potential targets for ODN therapy of glioma cells is extremely large, including genes coding for other examples of (1) growth factors and their receptors, (2) cellular proteases, kinases, and second messengers, (3) proto-oncogenes, and (4) factors and proteins important in cell cycle control and apoptosis, to identify but a few categories.

<table>
<thead>
<tr>
<th>Targets</th>
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<td><strong>Proto-oncogenes:</strong></td>
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<tr>
<td>c-sis</td>
<td>Nitta 1994</td>
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<td>c-erb B</td>
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<td>c-myb</td>
<td>Hall 1996</td>
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<td><strong>Growth Factors:</strong></td>
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<tr>
<td>Basic fibroblast growth factor</td>
<td>Morrison 1991, Murphy 1992, Behl 1993</td>
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<td>Transforming growth factor beta</td>
<td>Jachimczak 1996</td>
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<tr>
<td>Insulin-like growth factor 1</td>
<td>Resnicoff 1994</td>
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<td><strong>Other:</strong></td>
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Non-antisense Effects of ODN Treatment

Nonspecific and "paradoxical" (ie, opposite than those expected) effects of ODN treatment have been encountered even in the most simple in vitro systems. Nonspecific effects may in some cases be advantageous, such as inhibiting the migration of glioblastoma cells. The mechanisms for the nonspecific effects of ODNs could be related to (1) the structure of the ODN itself, (2) hybridization to DNA or mRNA other than the target sequence, with subsequent RNase cleavage, (3) binding to proteins or other molecules, and/or (4) ODN degradation products, which in themselves can affect cellular functions. Phosphorothioated ODNs have been shown to be inherently growth-inhibitory, particularly those having four adjacent guanosine bases.

As polyanions, ODNs have been shown to nonspecifically bind proteins such as bFGF, VEGF, PKC, and protein tyrosine receptors including the epidermal growth factor receptor. Phosphorothioated ODNs have also been reported to cause nonspecific induction of tumor necrosis factor, induction of Sp1 nuclear transcription factor binding activity, and inhibition of transferrin receptor expression. Nonspecific effects of phosphorothioated ODNs are usually encountered in the 20- to 50-µM range. Despite these nonspecific interactions, reports of ODN-mediated cellular toxicity have been rare.

ODN Pharmacokinetics and Delivery to the Brain

Studies of intravenous injection of unmodified (phosphodiester) ODNs have shown that the plasma half-life is approximately five minutes. The phosphorothioate modification produces a significant prolongation in plasma half-life to 30-60 minutes; steady-state plasma levels can be achieved with repeated daily intravenous injections. Phosphorothioate ODNs bind to serum albumin and alpha₂ macroglobulin and demonstrate two-phase pharmacokinetics. Regarding passage across the blood-brain barrier, most ODNs are negatively charged, and the molecular weight of an ODN of 14 bases is approximately 5 kDa. Animal studies of ODN biodistribution have shown that ODNs administered intravenously, subcutaneously, or intraperitoneally accumulate primarily in the liver, kidney, and other organs of the reticuloendothelial system, entering the brain only in minute quantities. Because of this, direct injection or osmotic minipump infusion into the CSF, brain parenchyma, or intracerebral tumors has been employed.

Studies of ODNs administered into the ventricles of the rat have shown that phosphodiester ODNs are rapidly degraded, whereas phosphorothioate ODNs are resistant to degradation and are cleared in a manner consistent with bulk flow. Phosphorothioate ODNs given for a week in this manner did not show evidence of
toxicity, yet penetrated the brain extensively and were taken up by astrocytes. Other investigators have confirmed the superiority of phosphorothioate ODNs for CNS administration, the cellular uptake and biodistribution of intracranially administered ODNs, and their apparent lack of adverse effects, 28,76-79 Transcription of a variety of different genes has been successfully blocked in nonneoplastic rat brain by means of direct ODN infusion into different regions of the brain. These genes play roles (as examples) in mediating traumatic brain injury, pain perception, thirst regulation, blood pressure elevation, memory functions, behavior and/or motor control. Some of the effects were seen with the administration of just a single ODN dose. ODNs may be more stable within the CNS than in other bodily compartments. In one report of a possible adverse effect, an ODN injected into rat brain was found to cause an inflammatory response, with induction of interleukin-6 expression. Liposome structures have been used to enhance ODN delivery to the CNS.

Potential Obstacles to Successful Clinical Use of ODNs in Oncology

In addition to the possible occurrence of nonspecific or even paradoxical effects in the antisense ODN treatment of tumor cells (as outlined above), other potential pitfalls to the clinical use of antisense ODNs certainly can be envisioned. While a possible advantage in terms of targeting for some diseases, accumulation of ODNs by the components of the reticuloendothelial system (when administered systemically) has the potential for producing adverse effects. In animal studies, elevation of liver enzymes, splenomegaly, immune stimulation, thrombocytopenia, prolongation of the activated partial thromboplastin time, and/or liver failure have been reported. Some of these side effects were found to be specific on ODN base sequence, backbone modification, and/or dosage schedule and could be avoided.

Even with acceptable toxicity, with adequate ODN entry into brain tumor cells (across or by circumventing the blood–brain barrier), and with demonstrated translation arrest of the target gene, successful treatment of malignant tumors is still likely to be problematic. Multiple genes may be important in determining the survival, proliferation, and invasiveness of cancer cells, and the expression of these genes may change over time in what is conceived to be a multistep process of malignant transformation. Malignant gliomas in particular are known to be highly heterogeneous as a group and even within a given patient’s tumor. Individual glioblastoma patients may express different sets of genes culminating in the malignant phenotype; furthermore, expression of different sets could coexist within different cells of the same tumor. Blocking one or more pathways to malignancy might simply result in the activation of an alternative means to continue to proliferate and invade adjacent brain.

In Vivo Use of ODNs Against Cancer: Preclinical Studies and Clinical Trials

Despite the theoretical obstacles to the successful use of antisense ODN therapy for cancer, ODN-induced down-regulation of tumor genes including c-myc, N-myc, c-myb, Ha-ras, c-raf, bcr-abl, PKC-alpha, PKA and NF-kappaB, has been achieved in several different animal cancer models. Yazaki et al reported the use of a phosphorothioate ODN directed against PKC-alpha that, when given intraperitoneally showed efficacy against U-87 (human glioblastoma) cells grown subcutaneously and intracerebrally in mice.

Clinical trials with ODNs are now proceeding for several different types of cancer as well as other diseases. Tumor genes that are being targeted include c-myc, bcl-2, PKC-alpha, p53 and c-raf. The results of the first phase I trials of a phosphorothioated ODN targeting p53 mRNA have been reported. No toxicity was observed in patients who received 0.05 to 0.2 mg/kg per hour ODN intravenously for 10 days. A phase I study for malignant brain tumors currently underway involves the systemic administration of an anti-PKC-alpha ODN (Isis/Ciba-Geigy), by the New Approaches to Brain Tumor Therapy Consortium based at The Johns Hopkins University.

Conclusions

Impressive advances have been made in molecular techniques over the past two decades. Such advances first led to the identification of potential targets for gene-targeted therapy (such as proto-oncogenes, second messengers, and growth factor receptors) and have now resulted in commercial production of molecules capable of specifically bind to these targets and disrupting their activity. Antisense ODN treatment of glioma cells can certainly be used to block gene expression in vivo; early results with ODNs administered in animal brain tumor studies have also been encouraging. As with almost any type of therapy, problems with the use of antisense ODNs for treating brain tumors and other CNS diseases (some of which have been discussed here) could be encountered. Potential difficulties related to the clinical use of ODNs, however, do not seem insurmountable.

Combination therapy with different ODNs or the use of ODNs in conjunction with conventional chemotherapeutic agents may be required to achieve therapeutic efficacy. Information pertaining to potential adverse interactions between conventional therapeutic agents and antisense ODNs is currently not available.

The idea of treating brain tumor patients with antisense ODNs continues to be attractive due to their theoretical specificity, relative ease of production through automated synthesis, and paucity (to date) of reported adverse effects. Even if toxic side effects are found, their occurrence will have to be balanced with the severity of the disease being treated. In the case of glioblastoma multiforme, if any efficacy of ODN treatment is shown, significant side effects might still be acceptable. Given the continued poor prognosis for patients with malignant brain tumors, preliminary results from phase I studies are being anxiously awaited.

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References


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