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HYPOTHESIS

Antisense Oligonucleotides as Specific Chemotherapeutic Delivery Agents: A New Type of Bifunctional Antisense

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Abstract. Antisense oligonucleotides (oligos) are linear strands of nucleotide bases synthesized in a sequence complementary to the protein-encoding mRNAs. These oligos inhibit the synthesis of specific proteins. In the *in vivo* and *in vitro* systems, oligos have demonstrated efficacy in inhibiting cancer cell growth through targeting protein growth factors, their receptors, transcription factors, or those that inhibit apoptosis. Since cancer growth is driven by the over expression of numerous proteins, it is naïve to believe that inhibition of single proteins would affect a cure, in most cases. Therefore, it is necessary that many gene products be suppressed. To enhance the efficiency of antisense therapy, oligos must be developed containing multiple (numerous) binding sites. In initial efforts, bispecific oligos have been evaluated which target more than one protein. While some of these bispecifics derive their activity from targeting proteins that share gene sequence homology, true bispecifics have been described which contain more than one binding site, which do not share sequence homology, and can target genes from even unrelated pathways. More complex branched (multispecific) oligos have also been proposed. Here we suggested that the concept of bispecific oligos should be expanded to include conjugated oligos as delivery agents. Since oligos have the ability to target only cells which over-express a specific protein, they also have potential to delivery and concentrate non-covalently bound chemotherapeutic (and toxic) agents, sparing normal cells from toxicity. Conjugated oligos would have both the (bispecific) capability for specific drug delivery and sequence specific inhibition.

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Abbreviations used: PSA, prostate specific antigen; PSMA, prostate specific membrane antigen.

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1. Introduction and Background

Antisense oligonucleotides (oligos) are linear strands of nucleotide bases synthesized in a sequence complementary to protein encoding mRNA. Translation arrest of the specifically targeted protein results from oligo:mRNA hybrid degradation by RNAse H, but may also be due to either DNA triplex formation first described in 1957 [1], protein binding interactions with growth factors [2], or other non-specific mechanisms which may not be dependent upon mRNA expression [3]. This approach was initially directed against proteins associated with viral replication [4], but more recently has been applied to those which regulate cancer progression, such as growth factors, their receptors, the apoptosis regulating protein bcl-2 or even translational factors (refer to ref. [5]). In order to increase biological action, several investigators have utilized single oligos having multiple mRNA binding activities. However it has recently been proposed [6] and demonstrated [7, 8] that more than one type of specificity could be included along a single linear stretch of oligo bases. Bispecific oligos simultaneously target two mRNAs and theoretically enhance RNAse mediated degradation by forming a larger and more complex hybrid structure. Bispecifics have the additional advantage of directing with equal efficiency (since both active sites are expressed in tandem sequences on the same linear molecule) two different complementary binding sequences to the same targeted cell. They also have the ability to target mRNAs encoding proteins associated with different biochemical or growth regulatory pathways, as well as to simplify the delivery process. Additional approaches used to enhance the biologic activity of bispecifics have utilized combination therapy with conventional chemotherapeutics [7, 9], and a branched form of antisense, which incorporates multiple ester linked oligos bound to a fat soluble backbone, has been proposed [10].

Specific delivery of antisense oligos to targeted tissues is often a limitation of this technology. Other than an intra-ocular delivery system employed for the treatment of CMV retinitis, specific targeting of a systemically administered oligo is limited, although progress has been made through innovative approaches, including those taken to target biotinylated oligos to prostate tumors utilizing monoclonal antibodies specific for prostate tissue expressed antigens. In these studies, two types of delivery vehicles were evaluated: One, consisting of a monoclonal with dual specificity for both prostate specific antigen (PSA) and biotin, directed biotinylated oligos to PSA expressing cells *in vitro* [11]; a second, employing monoclonals specific for either PSA or prostate specific membrane antigen (PSMA) to which avidin was conjugated onto Fc domains, delivered biotinylated (and radiolabeled) oligos *in vivo* [12].

A delivery system based upon the affinity between biotin and avidin forms the basis of many immunohistologic protocols, and there is no reason that it could not be developed as part of a delivery system, exploiting the sequence specificity of antisense oligos. Furthermore biotinylation of antisense has not been shown to adversely affect cellular transfection [13].

2. The Hypothesis

In this article we propose that antisense oligos can be employed as delivery vehicles for chemotherapeutics. Their non-covalent linkage to traditional chemotherapeutic agents would produce a new type of antisense, having bispecific activity. Taxol would be biotinylated through a spacer at position 7 as demonstrated by Byrd et al. [14]. Biotinylated antisense is already readily available from oligo manufacturers, where it is often included at the 5′ position. We hypothesize that avidin could be used to form a complex between biotinylated drugs (like Taxol) and biotinylated oligos for the treatment of cancer. Antisense oligos and Taxol differ greatly in both molecular weight as well as UV absorption. Purification of a fraction containing one oligo sequence and three Taxol molecules bound to an avidin carrier would maximize the delivery of the associated chemotherapeutic to a cell. In this scenario the oligo would primarily be used as a specific delivery vehicle. However, its significant ability to inhibit growth of the cancer cell would also contribute to the overall activity of the conjugate.

3. Evaluation of the Hypothesis

3.1. Synthesis

Biotinylated Taxol would be prepared as described by Byrd [14], using 7-β-alanytaxol synthesized according to the method of Nicolaou et al. [15].

Biotinylated antisense is available commercially, as is avidin. Biotinylated Taxol and biotinylated oligos, in an equal molar ratio, would be allowed to associate with avidin (provided at a rate limiting quantity) at room temperature. Since avidin has four biotin binding sites, five Taxol-avidin-oligo conjugates would be produced. Taxol and oligos differ greatly in both molecular weight and UV absorption. Therefore, separation of these complexes, which differ in ratio between the two bound biotinylated compounds, could be easily accomplished by molecular sieve chromatography. Those fractions containing bound antisense would be identified by absorption at 260 nm. Of the five complexed fractions expected (ranging from four parts Taxol/one part avidin to four parts oligo/one part avidin), four should consist of at least one bound oligo and absorb at 260 nm. The final eluted peak would be the lowest molecular weight conjugate, and would consist of avidin associated with four molecules of Taxol. The previous peak eluted (of greater molecular weight) and the last showing UV absorbance at both 260 nm (nucleic acid [oligo] absorbance) and 280 nm (protein [avidin] absorbance) should contain a single bound oligo with three bound Taxol residues. This would presumably be the desired peak, possessing oligo specificity and the greatest concentration of bound Taxol.

3.2. Testing Efficacy

The relative efficacy of this complex would be tested *in vitro* against PC-3 and LNCaP prostate tumor cell lines [7-9] as well as the MCF-7 breast cancer cells [16] as previously described.

4. Discussion

Antisense oligos have great potential for inhibiting synthesis of specific proteins associated with disease. However, their potential has yet to be fully realized. Further development could be enhanced through improvements in backbone, sugar and base modifications, enhanced delivery, combination therapy and multifunctional activity.

The concept of multispecificity was first proposed by Rubenstein [6] and later by Gleave [17] who directed a "multispecific" oligo against Bcl-2 and Bcl-X_L in combination with Paclitaxel against PC-3 prostate cancer cells producing a chemosensitization which allowed a 90% decrease in the amount of Paclitaxel required to attain a 50% level of inhibition. Oncogenex and Gleave have also evaluated a "multispecific" oligo, OGX-225, directed against both insulin-like growth factor binding proteins IGFBP-2 and IGFBP-5 to inhibit growth and induce apoptosis in U-87 glioblastoma cells [18]. This "multispecific" oligo was also tested against LNCaP and C4-2 prostate cancer cells, where it decreased cell survival and induced apoptosis by altering the IGF-1 signaling pathway [19]. All of these "multispecific" oligo activities are, however, based upon sequence homology. "Bispecific" oligos, as first proposed by Rubenstein [6], are distinctly different, having multiple binding sites and dual specificity not dependent upon sequence homology.

The bispecific antisense agent proposed here is a new type of bispecific oligo, which combines the inhibitory activity of a monospecific oligo with that of an associated chemotherapeutic agent. Its uniqueness is employing the antisense component primarily as a delivery vehicle for Taxol, would be concentrated in the cell at a three fold greater amount than the specific oligo. We present Taxol as a model drug for this procedure, but other drugs could be biotinylated as well. Other candidate agents must possess the ability to be biotinylated without compromising their activity, and it would aid in their preparation if they differed from the avidin carrier as well as the oligo in molecular weight, UV absorption spectra, etc. The choice of antisense oligo has not been discussed, but for prostate tumors those directed against TGF-α, EGFR, bcl-2, IGFBP-2 and -5 look promising [7-9, 17-19].

Other tumors, such as breast, could use a similar approach, employing Taxol or other suitable chemotherapeutic drug.

Antisense therapy could contribute to treatment of prostate or breast cancer patients by providing an additional tier of treatment based upon growth factor deprivation, regulation of apoptosis or modification of androgen (or estrogen) receptor expression.

Although it is likely that some of the ideas expressed in this manuscript have been discussed in the literature or have been considered within the pharmaceutical industry, we were unable to find them either evaluated or combined in this manner.

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