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OPTIMIZATION OF OLIGONUCLEOTIDES CHARACTERISTICS WITH TOPSIS

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Abstract. This paper focused on a new application of the TOPSIS method for the prediction and optimization of the oligonucleotides characteristics. This method has been used for these purposes as it has shown its efficacy for these analyses. This is the first time that it has been applied to the investigation of these biomolecules. The hypothesis in this paper was that the characteristics of these biomaterials would be optimized according to their structural differences. The obtained results showed that the stabilization of oligonucleotides would affect their ranking with TOPSIS when the stability of these biomolecules increased against enzymes in their structure. In other words, the oligonucleotides with less enzymatic degradation were ranked better with this method. This study showed the first application of this algorithm for the prediction and optimization of the oligonucleotides' characteristics. The results in this work revealed that the ranks of candidates depended on their distances from their ideal solutions. This showed that TOPSIS could be used as an appropriate method in the optimization of oligonucleotides as the rankings with this method would coincide with the data that concern the stability of these biomolecules against enzymatic degradation. The results of this work could be applied for the preparation of novel materials with applications in science and engineering.

Keywords: oligonucleotides, biomolecule materials, TOPSIS, prediction, decision-making process

Introduction

Antisense oligonucleotides are the biomolecules that hybridize to target sequences of messenger ribonucleic acid (RNA), resulting in the block of its translation into proteins [1–4]. During recent years, oligodeoxynucleotides (ODN) have been developed to target diseases caused by the undesired proteins generated from messenger RNA (mRNA). Hybridization of an ODN to mRNA can block gene expression by activating an enzyme, RNase H, which degrades the target site of mRNA [5–8].

Multidrug resistance (MDR) is a major problem in cancer therapeutics, which concerns the resistance of cancer cells to anti-cancer drugs [9–11]. The overexpression of the *mdr1* gene is a form of MDR. This gene encodes the transmembrane permeability glycoprotein (P-gp), which acts as a nonspecific efflux pump [12–15].

Previously, we investigated the antisense efficiency of several ODNs directed against *mdr1*-expressing cells. It was revealed that the minimal modifications of these biomolecules having a minimum of phosphorothioate linkages could protect them against serum and cellular exonucleases and endonucleases [16, 17].

Several motivations have initiated the current research work. First, it was interesting to perform the optimization and analysis of ODNs with the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS), a decision-making algorithm that would predict and determine the candidates' ranks

according to their distances from the best and worst alternatives. Secondly, the optimization and analysis of ODNs with TOPSIS to determine the impact of their stability against enzymatic degradation on their ranking have not been explored, yet. Finally, it was interesting to get the ranks of ODNs in order to compare their stability against enzymatic degradation in different cell lines.

To make the ODNs become effective biomolecules in order to attach to mRNA in cells, it would be necessary to deliver them to cells. Different types of delivery systems can be used for the internalization of ODNs to cells. In our previous work, we used cholesterol conjugated at the 5'-end of the ODN, a commercial transfectant (Superfect), or an amphotericin B derivative (AMA) for delivering the ODNs to the NIH-MDR-G185 cells [17].

The unmodified phosphodiester ODNs are rapidly degraded by serum or intracellular nucleases [18–21]. These biomolecules can be structurally modified to phosphorothioate ODNs in which nonbridging oxygen of the phosphodiester bond is replaced with sulfur. This can enhance their stability against enzymatic degradation [16, 17]. However, the nonspecific binding of phosphorothioate ODNs to proteins causes their nonantisense effect [22]. At high concentrations, these modified ODNs can inhibit the RNase H activity [23]. Therefore, we investigated the minimally modified phosphodiesters with minimal phosphorothioate sequences [16, 17]. Second-generation antisense ODNs, have the desirable properties of PS oligonucleotides, which is the resistance to nucleases and RNase H activation.

TOPSIS has been used previously for the optimization of materials [24–27]. To the author's knowledge, no investigation on the characteristic optimization of the antisense oligonucleotides against the *mdr1* with this algorithm has been performed, yet. The optimization and analysis of these biomolecules with TOPSIS is a novel application of this decision-making method.

The aim of this paper was to investigate the optimization and analysis of oligonucleotides with TOPSIS. For this, two different series of analyses were performed on two groups of ODNs. To the author's knowledge, this is the first comparative investigation of these biomolecules with TOPSIS. The results of the current paper can be used for the improvement of the use of these biomaterials in science and engineering.

Experimental approach

The antisense oligonucleotides investigated in this study were chosen from our previous studies for their effects against the *mdr1* gene. The ODNs in the current work were chosen according to their stability against enzymatic degradation revealed in our previous publications [16, 17].

In our previous work, the ODNs were delivered to the NIH-MDR-G185 cells by one of these delivery agents in our previous study: a cholesterol conjugated at the 5'-end of the ODN, a commercial transfectant (Superfect), or an amphotericin B derivative (AMA) [17].

Table 1 shows the list of the antisense oligonucleotides studied in the current paper with TOPSIS. All the partially or completely phosphorothioates were indicated with the stars at their nucleic bases for indicating the presence of sulfur in place of nonbridging oxygen in their bonds. The phosphodiester oligonucleotide, ODN-H, with no indicating star, did not have this modification in any of its bonds.

In the first series of analysis, the ODNs in Table 1, ODN-H, ODN-H1 and ODN-H2, investigated in our previous work [16], were indicated as the first, second and third candidates, or C₁, C₂ and C₃, in the matrices of TOPSIS, respectively.

Table 1

The list of the antisense oligonucleotides

Oligonucleotide	Sequence
ODN-H	5'-GATCCATCCCGACCTCGCGAAGC-3'
ODN-H1	5'-GAT*C*C*AT*CCCG*ACCTCGCGAAGC-3'
ODN-H2	5'-GATC*C*A*TC*CCG*ACCTCGCGAAGC-3'
AS-ODN	5'-C*C*A*T*C*C*C*G*A*C*C*T*C*G*C*G*C*T*C*C-3'
ODN1	5'-G*A*TCCATCCCGACCTCGCGAAGC-3'
S-ODN (control ODN)	5'-C*C*T*C*G*C*G*C*T*C*C*A*G*C*C*C*T*A*C*C-3'

The results that we obtained in that work in K562 cell lysate showed that ODN-H was completely degraded after 24 hours incubation and its $t_{1/2}$ was 1 hour 45 minutes. There was still 1 % ODN-H1 after this duration of incubation and its half-life ($t_{1/2}$) was 4 hours. 20 % ODN-H2 was intact after the incubation and its $t_{1/2}$ was about 10 hours. In other words, 100 % ODN-H, 99 % ODN-H1, and 80 % ODN-H2 were degraded after this duration of incubation, respectively.

In the second series of analysis, the ODNs in Table 1, AS-ODN, ODN1, and control ODN investigated in our previous work [17] were indicated as the first, second, and third candidates, or C_1 , C_2 and C_3 , in the matrices of TOPSIS, respectively. The three steps previously performed in our published paper with cholesterol conjugated ODNs, which were the samples that had a cholesterol conjugated at their 5' end, the ones delivered to cells with superfecta and the other ones delivered to cells with AMA [17], were considered in the matrices of TOPSIS. The efficiency of ODNs to block the P-gp expression was considered as a profit criterion in TOPSIS. In the investigation of the delivery of ODNs with cholesterol to cells, the remaining P-gp expressions with the internalization of AS-ODN, ODN1 and control ODN were 2 %, 6 % and 100 %, respectively. In other words, the efficiency of these ODNs to block this protein expression were 98 %, 94 % and 0 %, respectively. After their delivery with Superfect, the remaining P-gp expressions with the internalization of AS-ODN, ODN1 and control ODN were 15 %, 100 % and 100 %, respectively. Therefore, the efficiency of these ODNs to block this protein expression were 85 %, 0 % and 0 %, respectively. After their delivery with AMA, the remaining P-gp expressions with the internalization of AS-ODN, ODN1, and control ODN were 20 %, 40 % and 100 %, respectively. So, the efficiency of these ODNs to eliminate to block this protein expression was 80 %, 60 % and 0 %, respectively.

TOPSIS method

The TOPSIS algorithm with a code in Python presented on the GitHub website (<https://github.com/Glitchfix/TOPSIS-Python/blob/master/topsis.py>) was used for the analysis and optimization of ODNs with the same method described previously [24, 25, 26, 27].

Higher half-life values and lower percentage values of enzymatic degradation show more efficiency of ODNs to remain intact in cell lysate. Therefore, $t_{1/2}$ and enzymatic degradation in K562 cell lysate for these ODNs were considered as the profit criterion and cost criterion in the TOPSIS method, respectively. The half-life values of ODN-H, ODN-H1 and ODN-H2 were divided by 10. Therefore, the membership degree values of ODN-H, ODN-H1 and ODN-H2 calculated from their half-life values were 0.18, 0.40 and 1.00, respectively. The degradation percentages of ODNs were divided by 100 to result the membership degrees of these candidates to be used in TOPSIS. Therefore, the membership degree values of ODN-H, ODN-H1 and ODN-H2 calculated from their degradation percentages were 1.00, 0.99 and 0.80, respectively. These membership degree values were indicated in the values matrix in TOPIS.

The efficiencies of the ODNs to eliminate to block P-gp expression were divided by 100 to result their membership degrees in TOPSIS. Therefore, the membership degree values of AS-ODN, ODN1 and control ODN calculated from their efficiency to block the P-gp expression after their delivery with cholesterol were 0.98, 0.94 and 0.00, respectively; after their delivery with Superfect were 0.85, 0.00 and 0.00, respectively and after their delivery with AMA were 0.80, 0.60 and 0.00, respectively. These membership degree values were indicated in the values matrix in TOPIS.

Results and discussion

Table 2 shows the membership degree values of ODN-H, ODN-H1 and ODN-H2 as the first, second and third candidates, or C_1 , C_2 and C_3 , respectively.

Table 2

The membership degree values of ODN-H, ODN-H1 and ODN-H2

Candidates/Criteria	$t_{1/2}$	Enzymatic degradation in K562 cell lysate
C_1	0.18	1.00
C_2	0.40	0.99
C_3	1.00	0.8

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Tables 3 and 4 show the weight values applied for each criterion of ODN-H, ODN-H1 and ODN-H2 and the criteria matrix, respectively.

Table 3

Weight values applied for each criterion of ODN-H, ODN-H1 and ODN-H2

Alternatives/Values	$t_{1/2}$	Enzymatic degradation in K562 cell lysate
C_1-C_3	0.5	0.5

Table 4

Criteria matrix for ODN-H, ODN-H1 and ODN-H2

Alternatives/Values	$t_{1/2}$	Enzymatic degradation in K562 cell lysate
C_1-C_3	true	false

Table 5 shows the distances from the best alternative and the worst alternative, the similarity coefficients as well as the ranking of ODN-H, ODN-H1 and ODN-H2.

Table 5

The distances from the best and worst alternatives, the similarity coefficients and ranking of ODN-H, ODN-H1 and ODN-H2

Candidates	d_i^*	d_i^-	CC_i	Ranking
C_1	0.38051653	0.00000000	0.00000000	3
C_2	0.28093158	0.10078265	0.26402645	2
C_3	0.00000000	0.38051653	1.00000000	1

ODN-H2 with the highest stability against enzymatic degradation had the first position in the ranking with TOPSIS. ODN-H1 and ODN-H with less stability and the worst stability had the second and third ranking positions, respectively.

Table 6 shows the membership degree values of AS-ODN, ODN1 and control ODN as the first, second and third candidates, or C_1 , C_2 and C_3 , respectively.

Table 6

The membership degree values of AS-ODN, ODN1 and control ODN

Candidates/Criteria	Delivery of cholesterol ODNs	Delivery with Superfect	Delivery with AMA
C_1	0.98	0.85	0.80
C_2	0.94	0.00	0.60
C_3	0.00	0.00	0.00

Tables 7 and 8 show the weight values applied for each criterion of AS-ODN, ODN1 and control ODN and the criteria matrix, respectively.

Table 7

Weight values applied for each criterion of AS-ODN, ODN1 and control ODN

Alternatives/Values	Delivery of cholesterol ODNs	Delivery with Superfect	Delivery with AMA
C ₁ -C ₃	0.5	0.5	0.5

Table 8

Criteria matrix for AS-ODN, ODN1 and control ODN

Alternatives/Values	Delivery of cholesterol ODNs	Delivery with Superfect	Delivery with AMA
C ₁ -C ₃	true	true	true

Table 9 shows the distances from the best alternative and the worst alternative, the similarity coefficients as well as the ranking of AS-ODN, ODN1 and control ODN.

Table 9

The distances from the best and worst alternatives, the similarity coefficients and ranking of AS-ODN, ODN1 and control ODN

Candidates	d _i ⁺	d _i ⁻	CC _i	Ranking
C ₁	0.00000000	0.48999142	1.00000000	1
C ₂	0.34007641	0.30535511	0.47310226	2
C ₃	0.48999142	0.00000000	0.00000000	3

AS-ODN with the highest half-time value and the lowest nuclease degradation was ranked in the first position by TOPSIS. ODN1 and control ODN with less half-time values and the lower nuclease degradation had the second and third-ranking positions, respectively. As ODN1 was more stable against the cell lysate nucleases than control ODN, this first one was ranked in the second position, whereas this last one had the last ranking position.

The results obtained in the current work showed that TOPSIS has been an appropriate method in the optimization of ODNs as the rankings with this method have coincided with the data concerning the stability of these biomolecules against enzymatic degradation.

Several biomolecules and nanomaterials have been assembled with ODNs, previously [28–32]. However, they have not been investigated and optimized according to their characteristics with TOPSIS. During recent years, the physicochemical [33–37], rheological [38–41], and biological [42–45] properties of many biomolecules and nanomaterials have been studied. The characteristics of these materials are related to their preparation procedures [46–50]. Some materials and processes have been optimized with TOPSIS [51–53]. However, no comparative analysis has been done on their optimization, yet. This method has found diverse applications in different fields of science [54–57]. It has also been applied in combination with other analysis methods [58–62]. More investigations would be required to determine how the optimization of ODNs would be affected when ODNs were assembled with these biomolecules and nanomaterials.

Conclusions

The current paper aimed to investigate the optimization and analysis of oligonucleotides with TOPSIS. The TOPSIS method used in this work had the same procedure as the ones in the previous

investigations with This algorithm. ODNs selected in the current work have shown their efficiency against enzymatic degradation. The results obtained with TOPSIS showed that the candidates with higher stability would have a better ranking position than other candidates with TOPSIS. Moreover, the ranking results with these algorithms coincided with our previous data concerning the efficiency of these biomolecules. More investigations would be required for the optimization of ODNs assembled with other biomolecules as well as nanomaterials. The results obtained in the current work can be used in the development of these materials. This can give a new insight for their further applications in sciences and engineering.

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