ABSTRACT
Antisense oligonucleotides (ODNs) technology is one of the important approaches for the sequence-specific knockdown of gene expression. ODNs have been used as research tools in the post-genome era, as well as new types of therapeutic agents. Since finding effective target sites within RNA is a hard work for antisense ODNs design, various experimental methods and computational approaches have been proposed. For better sharing of the experimented and published ODNs, valid and invalid ODNs reported in literatures are screened, collected and stored in AOBase. Till now, ~700 ODNs against 46 target mRNAs are contained in AOBase. Entries can be explored via TargetSearch and AOSearch web retrieval interfaces. AOBase can not only be useful in ODNs selection for gene function exploration, but also contribute to mining rules and developing algorithms for rational ODNs design. AOBase is freely accessible via http://www.bioit.org.cn/ao/aobase.

INTRODUCTION
With the ability to selectively down-regulate the expression of genes, antisense oligonucleotides (ODNs) have been widely used in gene function determination, drug targets validation and pathways discovery (1–3). Recently, ODNs also serve as specific and efficient inhibitors for systematic loss-of-function analysis of miRNA (4,5). On the other hand, ODNs can be effective therapeutic agents. Several antisense compounds for disease treatment have been evaluated in clinical trials with promising results (6,7). However, the successful use of ODNs is somewhat limited since only a small number among all the possible antisense ODNs against a given target RNA show effective suppression of the target gene in living cells (8,9). It is commonly accepted that the selection of sensitive sites in target RNA is of great importance for ODNs efficiencies. Various experimental approaches to identify promising local target sites have been presented in recent years (9–11). There has also been much interest in computational approaches to select target sites of ODNs, which get prominent advantages over experimental protocols in throughput, cost and efficiency (12–15).

In fact, for the researchers who use ODNs as gene expression modulation tools to explore gene functions or molecular networks, it is not necessary to screen ODNs targeting specific mRNA if they could find some with enough activity in literatures or database, considering that experimental ODNs screening methods are time consuming and expensive. However, for the researchers whose efforts focus on the development of in silico antisense ODNs design methods, information about both valid and invalid ODNs are of same value. Rules for rational target site selection can be mined from these positive and negative cases. Therefore, if the related data for ODNs are collected together, there would be obvious benefit for ODNs users and designers.

Three ODNs resources have been reported till now. The first public ODNs database named ODNBase was developed five years ago by Giddings et al. (16). Unfortunately, it cannot be accessed at present. Another database is a non-public database maintained by Isis Pharmaceuticals (17), which stores the data from the experiments performed at the corporation and is not publicly available. There is also a data list named AOdb, which is freely available for ODNs prediction algorithm research (13), containing basic descriptions of 315 ODNs. The AOBase we describe here is a database developed for both ODNs selection and ODNs design. It can be freely accessed at http://www.bioit.org.cn/ao/aobase. A more comprehensive dataset has been elaborately screened and constructed, and two retrieval tools have been developed. AOBase can be used to select effective ODNs for gene expression modulation, and can also contribute to mining rules and developing algorithms for rational ODNs design.

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The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors

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DATABASE CONTENT AND IMPLEMENTATION

Criteria for data collection

Antisense ODNs stored in AOBase are all collected from published literatures. There have been a lot of techniques used in antisense studies, such as various chemical modification methods and different types of efficacy assay. Since the database is not specifically constructed for some ODNs effectiveness prediction system, the data selection criteria is not as strict as that used in efficacy prediction research (12,15). The reference selection criteria are (i) at least one scrambled or mismatched sequence was used as control; (ii) efficacy of ODNs was carried out against controls via \textit{in vitro} assay at RNA or protein level; and (iii) efficacy was presented as a percentage of the control level of the target expression. The species of target RNAs are not restricted (Figure 1A). Presently, the database maintains \( \sim 700 \) ODNs against 46 different RNA molecules.

To keep in line with most of the researches on drug design, the ODNs efficacy in AOBase is transformed into \( (1 - [\text{control expression}]) \). The distribution of ODNs efficacy in the database is relatively uniform (Figure 1B). Considering that the selected ODNs were tested under different experimental conditions, some supplementary descriptions were also included in the database, such as chemical modifications employed for ODN synthesis, assay type used to measure the activity, concentration applied in test, etc.

Target region of ODNs

Target region selection is usually considered in ODNs design. Regions surrounding translation initiation codon are often chosen as target sites, since they are essential for gene expression and generally free from secondary structure (9). In the opinion that cleavage in 3'-untranslated regions (3'-UTRs) will lead to rapid degradation of mRNA, the 3'-UTR of mRNA is also targeted frequently (9).

Target region of each ODN is annotated in AOBase. Bases of target RNA at different regions are marked with different colors shown in a detailed description page of each ODN (Figure 2G). The distribution of target regions in AOBase is shown in Figure 1C.

Local structures of target sites

Among the factors that influence the activity of ODNs, the local secondary structures of target RNA are well known to play a very significant role in determining ODNs efficacy \textit{in vitro} (1,9,18). Although it is commonly accepted that computation-based structure models of long RNA molecules are not yet reliable enough to represent the RNA structure in living cells (19), the computational predicted structure of target RNA, especially the minimum free energy (MFE) structure, is still of particular importance in ODNs design strategies (20–22). Considering that single-stranded regions of RNA molecules play many important roles in RNA–RNA, RNA–DNA and RNA–protein interactions, the single-stranded probability profile, which is a kind of probabilistic representation of RNA structure, is also used in computer-aided ODN selection (23,24).

Both these two structural representations of ODN target region addressed above can be found in AOBase to help the structure–efficacy relationship exploration. For each target RNA, all the secondary structures within 5\% of the computed MFE were predicted by MFold (25). The upper limit on the number of computed structures is 50. The corresponding single-stranded probability profile was estimated from the MFold ss-count file. In the detailed description page of ODN, the target site is highlighted in MFE structure illustration, while the single-stranded probability profile of target is plotted as waveform (Figure 2G).

Figure 1. Overview of target and ODNs in AOBase. (A) Species distribution of target RNA molecular. (B) Efficacy of ODNs. (C) Target regions of ODNs.
WEB RETRIEVAL INTERFACES

To afford convenience for ODNs users and designers, two web retrieval interfaces named ‘Target Search’ (Figure 2B) and ‘AO Search’ (Figure 2E) have been developed. The search engine is written in PHP script with SQL code embedded.

Through ‘Target Search’ interface, users can query target RNA by name, accession number or only imprecise descriptions. The matched target RNA molecules will be displayed if search result is not empty (Figure 2C). With hyperlinks, users can explore all the ODNs targeting against these RNA molecules (Figure 2D). This interface would be helpful to the users who want to select effective ODNs to inhibit the expression of some genes.

‘AO Search’ interface allows users to search ODNs with several combined parameters, including activity measured, concentration applied, target region and motifs in oligo sequence. A number of sequence elements reported by Smetters et al. (26), Tu et al. (27) and Matveeva et al. (28), which may be positively (i.e. CCAC, TCCC, ACTC, GCCA, CTCT) or negatively (i.e. ACTG, GGGG, TAA, CCGG, AAA) related with ODNs effectively, were added to an item list for choice. Users can also search for ODNs with custom-defined sequence motifs. The target region parameter can be set to 5' - and 3'-UTR, CDS, initiation codon, intron, regions between exon and intron, or ncRNA. A list of ODNs matching the searching criteria will be provided to users (Figure 2F). AOSearch retrieval interface provides users the possibility of additional data mining and automatic datasets generation for construction of efficacy prediction system.

Each entry in AOBase has a detailed descriptions page (Figure 2G) in which the sequence, references, assay type, structure of target site and other related information is presented. To facilitate data collection and analysis, raw information about ODNs listed in search result pages (Figure 2D and F) can be selectively downloaded into a text file in CSV format, which can be easily processed by MS Excel and other programs.

To maintain an up-to-date resource, a data submission page (Figure 2H) has been developed. Researchers are encouraged to submit their ODN data to AOBase as soon as their paper is published. We would manually check the submission based on our data collection criteria mentioned above to determine its acceptability.

FUTURE WORKS

The factors which influence the potential of ODNs are complex and poorly understood till now. There are still many challenges in the studies of computational ODNs design. Compared with most of the other bioinformatics research problems, these studies are far from ‘data rich’, and besides, the data collected from published literatures are variable due to the diversity of experiment methods. To provide the basis for the development and test of ODNs design algorithms, our further works will focus on dataset enlargement and retrieval tools improvement. More ODN data with quality control and more powerful data filter tools which help to generate homogeneous dataset will be integrated into the database.
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