

SCIENTIFIC REPORTS



OPEN

siRNAmod: A database of experimentally validated chemically modified siRNAs

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Received: 02 October 2015

Accepted: 21 December 2015

Published: 28 January 2016

Small interfering RNA (siRNA) technology has vast potential for functional genomics and development of therapeutics. However, it faces many obstacles predominantly instability of siRNAs due to nuclease digestion and subsequently biologically short half-life. Chemical modifications in siRNAs provide means to overcome these shortcomings and improve their stability and potency. Despite enormous utility bioinformatics resource of these chemically modified siRNAs (cm-siRNAs) is lacking. Therefore, we have developed siRNAmod, a specialized databank for chemically modified siRNAs. Currently, our repository contains a total of 4894 chemically modified-siRNA sequences, comprising 128 unique chemical modifications on different positions with various permutations and combinations. It incorporates important information on siRNA sequence, chemical modification, their number and respective position, structure, simplified molecular input line entry system canonical (SMILES), efficacy of modified siRNA, target gene, cell line, experimental methods, reference etc. It is developed and hosted using Linux Apache MySQL PHP (LAMP) software bundle. Standard user-friendly browse, search facility and analysis tools are also integrated. It would assist in understanding the effect of chemical modifications and further development of stable and efficacious siRNAs for research as well as therapeutics. siRNAmod is freely available at: <http://crdd.osdd.net/servers/sirnamod>.

RNA interference (RNAi) phenomenon was described by A. Fire and coworkers in *Caenorhabditis elegans*¹. They injected double stranded RNA that potentially and specifically interfere with the endogenous gene at mRNA level leading to its silencing¹. RNA silencing pathway utilizes dicer to process long double stranded RNA (dsRNA) to 19–21 nucleotide small interfering RNAs (siRNAs) with 2-nucleotide unphosphorylated 3' overhangs². RNA induced silencing complex (RISC) incorporates siRNA antisense strand, resulting in cleavage of cognate mRNA target³ as shown in Fig. 1.

siRNAs are explored extensively in gene silencing experiments and therapeutics development⁴. However, their therapeutic usage is hampered due to many factors e.g. susceptibility to ribonucleases digestion, short biological half-life, lack of proper delivery vehicle, cellular uptake, immune-stimulatory effect, non-specific gene targeting and toxicity^{5,6}. These limitations are due to the inherent physicochemical properties of siRNAs that includes short and stiff structure, high charge density (poly-anionic), hydrophilic and liability to nuclease cleavage^{5,6}. To mitigate these restraints, chemical modifications in siRNAs have been substantially investigated⁷.

Synthetic oligonucleotides were used in the late 1970's for gene inhibition known as antisense technology⁸. Modified nucleoside phosphoramidites are used in solid phase chemical synthesis of siRNA which allow site-specific incorporation of chemically modified nucleotide moiety at specific positions within the siRNA⁹. Chemical modifications on siRNA are categorized based on component of nucleotide modified as ribose, base or phosphate and change in properties like biological activity, thermodynamic stability and nuclease resistance⁷. Chemical modifications on sugar moiety e.g. locked nucleic acids (LNA), unlocked nucleic acids (UNA)^{10,11}, 2'-deoxy, 2'-O-methyl¹², 2'-fluoro, 2'-methoxyethyl, 2'-aminoethyl, were tested for RNAi activity^{13,14}. In LNA, methylene bridge is created between 2'-oxygen and 4'-carbon to increase the RNAi activity favorably^{15,16} and also improves nuclease resistance¹⁷. UNA are open ring derivatives of ribose without C-2' and C-3' carbon-carbon bond but structurally mimic the RNA when incorporated into duplex¹⁸. Ribose 2'-OH and phosphorothioate modification increases stability of siRNA^{19,20}. Besides the 2'-OH was not found to be essential for RNAi activity²¹. However, fluorine modification at 2'-OH on different nucleotide positions of siRNA maintains silencing

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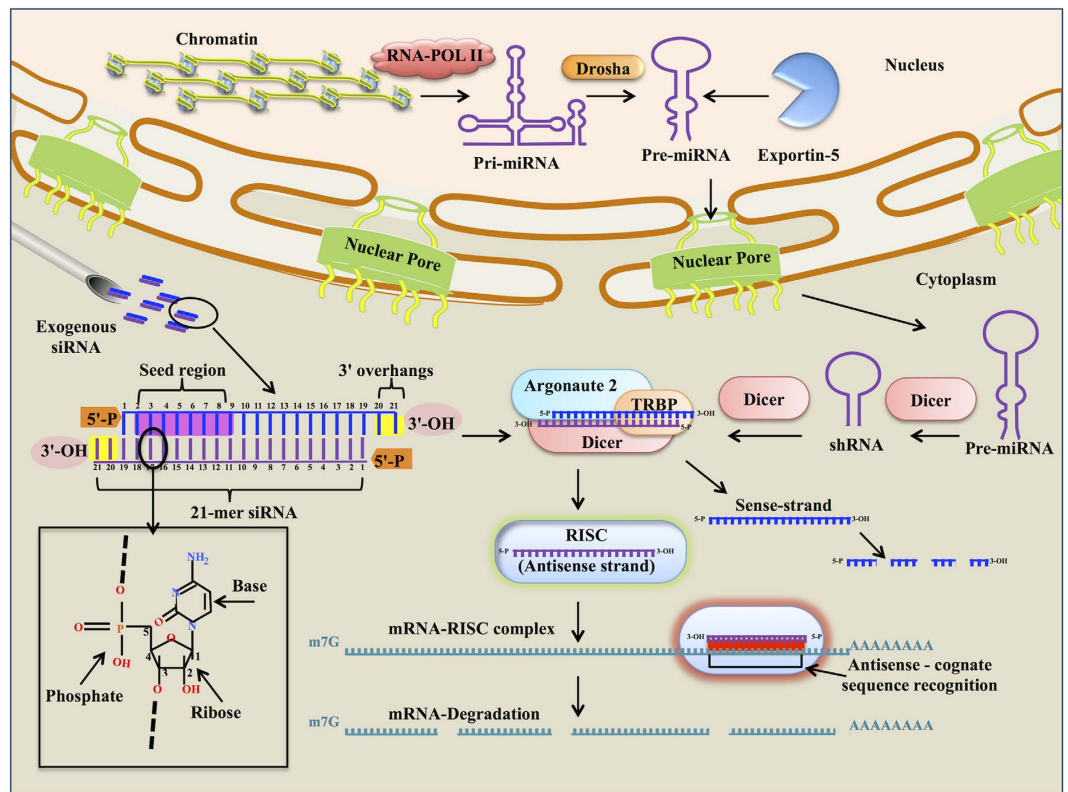


Figure 1. Cartoon representation of RNAi mechanism, siRNA molecular structure and its entry in RNAi pathway.

potency as well as increases thermal stability^{22,23}. Further, researchers carried out various chemical modifications in different combinations and tested them for RNAi activity^{13,14}. Also 2'-O-aminoethyl addition on passenger strand (sense strand) at 3'-end enhanced RNAi activity of siRNA duplex due to asymmetry in thermodynamic stability²⁴.

Base modifications have been used in various studies e.g. hypoxanthine, 2,4-difluorotoluene, dihydrouridine, 2'-thiouridine, pseudouridine^{12,25,26} etc. Modification in this component can contribute towards better understanding of off-target effects as well as gene silencing mechanism²⁷. Besides, phosphate modifications were also employed in different studies to enhance the properties of siRNAs^{28,29}. These modifications include phosphorothioate, boranophosphate, peptide nucleic acids (PNA)^{28,29} etc. For instance phosphorothioate enhances the stability, uptake and biodistribution of the siRNA^{30,31}. Large-scale screens and high throughput platforms using different permutation and combinations of chemical modifications were explored to check their effect^{24,32}. Native (un-modified) siRNA show immunological activation *in vitro* to various degrees, while certain chemical modifications (2'-O-methyl) of siRNA avoid immunological effects³³. Chemical modifications may be useful to overcome the obstacle of delivery of siRNAs without compromising their gene silencing activity⁵.

As discussed, next generation siRNA technology will be defined by the chemistry of modifications on siRNAs to greater extent. But we currently lack the platform to explore the complete spectrum of chemical modifications synthesized and plotted with relation to siRNAs so far. To describe the rules governing the performance of these cm-siRNAs, we need to have birds-eye view on the entire set of chemical modifications. Furthermore, positions of modifications on sense and antisense strands, modified nucleotide component, chemical features like size, functional groups, etc. need to be investigated thoroughly. Other associated experimental details like target gene, transfection method, cell line or organism used are very important and needs to be considered before further investigation. Additionally, the data needs to be present in the format that can be easily retrieved and may be utilized for further computational analysis.

Many repositories for native (unmodified) siRNA are available in the literature like siRecords³⁴, siRNAdb³⁵, human specific siRNA database (HuSiDa)³⁶, viral siRNAs (VIRsiRNAdb)³⁷ and HIV specific siRNA (HIVsirDB)³⁸. Also for naturally occurring modified RNA, there is a database namely RNAMDB³⁹. The latter provides comprehensive listing of the post transcriptionally modified nucleotides occurring naturally as in messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). But no dedicated repository has been developed yet for cm-siRNA despite their usefulness. Therefore, we developed siRNAmold that will serve as the central repository of the experimental records of chemical modification of siRNAs.

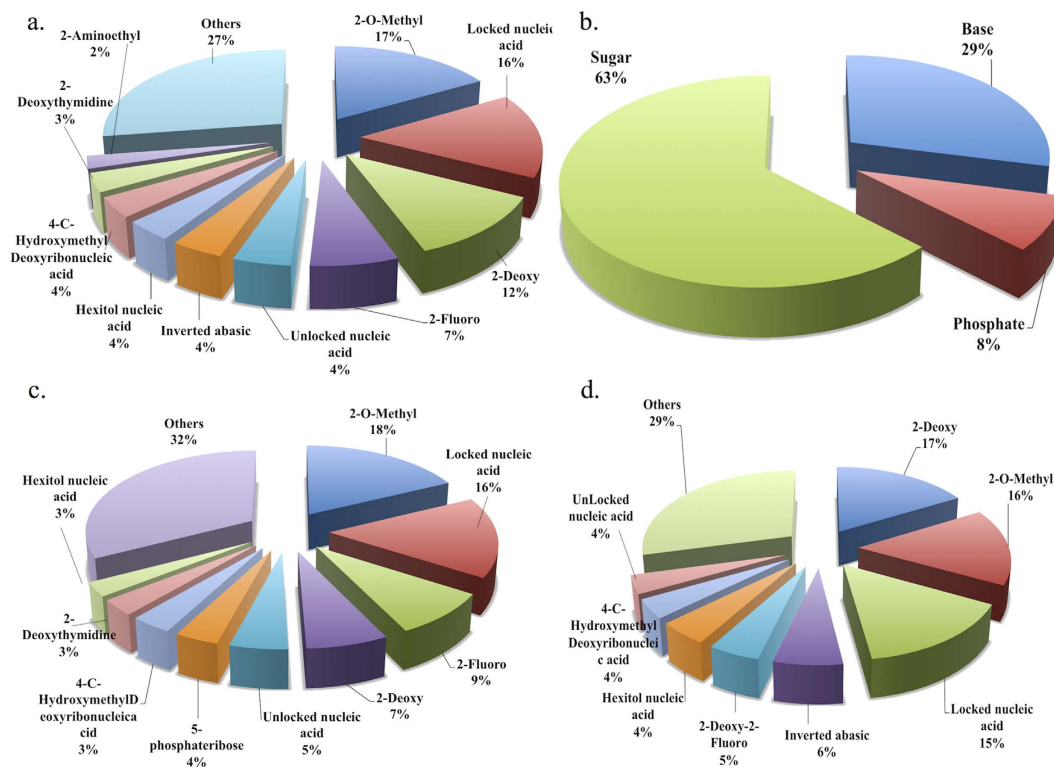


Figure 2. Chemical modification statistics pie charts (a) Overall siRNA modifications, (b) nucleotide component based (sugar, base phosphate), (c) Antisense strand modification distribution and (d) Sense strand modification distribution.

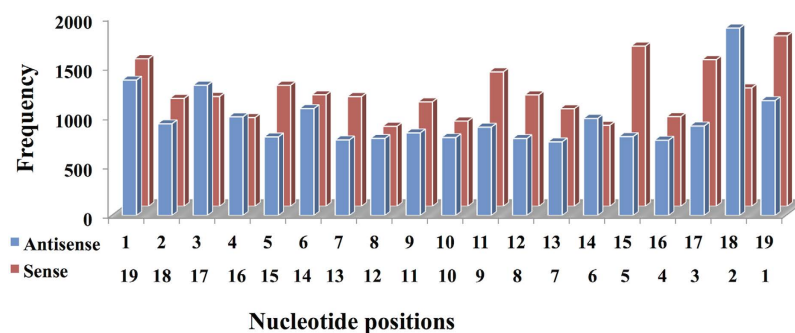


Figure 3. Reverse complement based chemical modification of siRNA position wise on sense and antisense siRNA sequences.

Results

Database statistics. siRNAmoD contains 4894 experimentally validated cm-siRNA entries. 128 unique chemical modifications incorporated on various components of nucleotides are archived. The overall statistics of the chemical modifications is depicted in Fig. 2. Chemical modifications distribution is illustrated in Fig. 2a. Majority of the reported modifications executed are 2'-O-methyl, locked nucleic acid, 2'-deoxy, 2'-fluoro and unlocked nucleic acid with 17%, 16%, 12%, 7%, and 4% respectively. Both 2'-O-methyl and 2'-deoxy are natural RNA modifications. The percentage distribution of natural and non-natural chemical modifications of cm-siRNAs is shown in supplementary Fig. S1. The individual distribution of chemical modifications for antisense and sense siRNA strands is represented in Fig. 2c,d correspondingly. Main modifications are directed on ribose with 63% followed by base and phosphate modifications with 29% and 8% respectively (Fig. 2b). The chemical modifications of the base moiety include hypoxanthine, 2,4-difluorotoluene, boron cluster. While phosphate modifications contain phosphorothioate, boranophosphate, peptide nucleic acid, etc.

Complementarity based sense and antisense distribution of chemical modifications is shown in Fig. 3. Statistical trend of cumulative distribution and combinations of different chemical modifications is represented in supplementary Fig. S3 and S4. Different experimental methods like luciferase assay, RT-PCR, northern blotting etc. were used to validate cm-siRNAs. Most common method to evaluate the efficacy was luciferase

a. SMDBid

| SMDBid | siRNA (sense/antisense) 5'>3' | Modification (sense/antisense) | Position (sense/antisense) | Modified component (sense/antisense) | Efficacy (%) | Reference |
|--------|---|------------------------------------|----------------------------|--------------------------------------|-------------------------------------|-----------|
| SM0001 | GACGUAAAACGGCCACAAGUUC ACUUGUGGCCGCUUACGUCGC | 2-Aminoethyl 2-O-Methyl | 18 2 | Sugar Sugar | 69.5 percent target mRNA inhibition | 19282453 |
| SM0002 | GACGUAAAACGGCCACAAGUUC ACUUGUGGCCGCUUACGUCGC | 2-Guanidinoethyl 2-O-Methyl | 18 2 | Sugar Sugar | 76.8 percent target mRNA inhibition | 19282453 |
| SM0003 | GACGUAAAACGGCCACAAGUUC ACUUGUGGCCGCUUACGUCGC | Hexitol nucleic acid 2-O-Methyl | 2,8,14,17 2 | Sugar Sugar | 54.2 percent target mRNA inhibition | 19282453 |

b. SM0001 Chemically modified siRNA information

| Field | Sense | Antisense |
|---|-------------------------------------|-----------------------------|
| siRNA chemical modification | 2-Aminoethyl | 2-O-Methyl |
| siRNA modification types | 1 | 1 |
| Overall number of modifications | 1 | 1 |
| Position of modifications | 18 | 2 |
| Modification on sugar or base or phosphate | Sugar | Sugar |
| SMILES (Click to view structure & nomenclature) | <chem>NCCOC(C)OC(C)C1O</chem> | <chem>COC(C)OC(C)C1O</chem> |
| siRNA Sequence | GACGUAAAACGGCCACAAGUUC | ACUUGUGGCCGCUUACGUCGC |
| siRNA length base-pair | 21 | 21 |
| siRNA name in paper | DO003 | DHARM1 |
| Biological activity | 69.5 percent target mRNA inhibition | |
| Experiment used to check activity | Luciferase reporter assay | |
| Melting temperature (°C) | NA | |
| Target gene | EGFP gene | |
| siRNA concentration | 10 nM | |
| Cell or Organism used | HeLa cells | |

c. Information of chemical modification

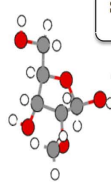
| | |
|--------------------|---|
| Structure |  |
| Name/Synonym | 2-Methoxy; 2-Methoxyribose; 2-O-methylribose; 2-O-Methyl |
| Systematic Name | (3R,4R,5R)-5-(hydroxymethyl)-3-methoxyoxolane-2,4-diol |
| InChI | InChI=1S/C6H12O5/c1-10-5-4(8)(3(2-7)11-6(5)9/h3-9H,2H,1H3/4,5-,6)/m1/s1 |
| Canonical SMILES | <chem>COC1C(O)OC(CO)C1O</chem> |
| Source | Naturally found RNA modification |
| Modified Component | Sugar |

Figure 4. Display of search output with cm-siRNA details (a) Result output of search or browse (b) Details page of individual entry (c) Structure and other chemical information of modification.

assay. The cm-siRNA stability was checked using melting temperature experiments or thermodynamic stability. Transfection methods used on different cell lines mainly utilized lipid based reagents viz lipofectamine and Interfer (Polypus-Transfection). Percentage efficacy ranges from 0–100. However, negative efficacy values were also reported with respect to control taken in a particular experiment. Therefore, for the sake of simplicity, we have reported negative efficacies as zero.

For experimentation, both *in vivo* and *in vitro* systems were reported in the literature. Commonly used cell lines were HeLa, HEK 293, Hepa 1–6, Vero, SH-SY5Y etc. Further, *in vivo* system includes Balb C mice, C57BL/6 mice etc. Pie chart depicting their distribution is presented in supplementary Fig. S5. 128 unique chemical modification structures used to modify the siRNA nucleotides are organized in the form of list with hyperlinks to information of individual modification. Here, we have combined the similar types of modifications into single categories as synonyms to represent the same or similar modifications reported in different studies.

Database browsing. siRNAmod browsing option helps user to surf via these five categories: i) modification in sense strand, ii) position of modification in sense strand, iii) modification in antisense strand, iv) position of modification in antisense strand and v) reference. Modifications that include more than one chemical moiety on same strand are separated by the asterisk (*) symbol. “0” modification in the fields of sense or antisense strand indicates that the modification is present on the other strand.

Simultaneously, facility to navigate 128 chemical modifications is also provided. This offers user an overview of chemical moieties used for modification to modulate siRNA properties. Clicking on the individual modification, structure and related chemical information about the chemical moiety is displayed via jmol⁴⁰.

Database search. There are two search options provided (i) basic search (ii) structure based search. For basic search, user can enter keyword in query-box and find relevant information. User can choose exact or containing operators. As indicated from the name, “exact” option is the strict search mode, which finds the exact term that user provides while “containing” is lenient search option and provides wider results. The output is displayed in Fig. 4a. On clicking individual Id, user can look for its detail. Further, structure of the chemical modification can be viewed by clicking on the SMILES link as provided (Fig. 4b,c). The result output can be filtered and sorted by clicking or writing on the column headers.

For structure drawing and search, MarvinSketch tool is provided. Chemical structures can be hand drawn and used to search for their presence or absence in the database. Step by step instructions for searching database and its output are displayed using screen shots in “How to use” menu on the web server.

Tools. siRNAmod-BLAST provides similarity search option to look for siRNA sequences against the database. This will help the user to know whether similar siRNA sequences are already reported in database or not.

siRNAmoD-Map enables the user to map cm-siRNAs reported in the database against a particular nucleotide sequence. In addition, we have also provided submit option for new chemical entries by various researchers. Further help page and other related links are provided.

Implementation. Database front end is developed using Perl, PHP, JavaScript HTML and CSS. It is maintained on MySQL server using LAMP software solution. The resource is hosted on IBM SAS × 3800 machine. siRNAmoD server and its integrated tools are compatible with all browsers like Google Chrome, Mozilla Firefox and Safari. However, structure based search tool (MarvinSketch) needs java to be enabled on the browser.

Discussion

siRNAs are being actively tested as new potential therapeutics against various disorders as well as pathogens^{37,41,42}. More than 20 siRNA based therapeutics are currently at different phases of clinical trials⁴³. Examples of such cm-siRNA drugs are SPC2996, EZN3042 and SPC3649 for leukemia, solid tumors, and Hepatitis C virus (HCV) infection respectively⁴³. Chemical modifications remain the critical aspect for development of siRNA therapeutics to reduce their inherent limitations^{6,44}. For example, 2'-fluoro, 2'-O-methyl, 2'-deoxy, unlocked nucleic acid, 2'-hydroxy and phosphorothioate modification increase siRNA serum stability^{19,20}. Likewise, certain nucleotide motifs in siRNA sequence as UA and CA upon modification with 2'-O-methyl or 2'-fluoro enhances the nuclease resistance⁴⁵. Locked nucleic acid, 2'-O-methyl, 2'-fluoro moieties thermodynamically stabilize siRNA ends while unlocked nucleic acid, phosphorothioate, ethylamino, dihydrouracil modifications destabilize them⁴⁶. Stabilizing the 5'-end and destabilizing the 3'-end of passenger strand enhances the potency of siRNA and vice versa⁴⁶. siRNAs of length 19 to 21 base pairs are less immunogenic than longer ones⁴⁷. Modifications like 2'-thiouridine, 4'-thiouridine, 2'-deoxyuridine reduce Protein kinase R (PKR) activation⁴⁸ while 2'-fluoro, 2'-deoxy, and 2'-O-methyl modifications abolish Toll like receptor (TLR) interaction preserving silencing activity even upon heavy modification⁴⁶.

Various comprehensive studies in this field have been conducted which employed new strategies and methods. Bramsen and coworkers²⁴ utilize 21 different chemical modifications on various positions of sense and antisense strands. Both strands were then combined to generate permutations and combinations of cm-siRNAs. Butora *et al.* employed walkthrough method^{13,32}, which replaces single or multiple nucleotides in siRNA with different modifications sequentially from one end to another and checking their biological impact. Further effect on enhanced efficacy due to chemical modifications was tested using different siRNAs concentrations⁴⁹. Further, compatibility of RNAi protein machinery with cm-siRNA format has been investigated^{50,51}. Peel *et al.* examined target degradation efficacy of small molecules conjugated to siRNAs⁵². Similarly, other groups also studied terminal modifications like 5'-phosphate⁵³ or serinol modification⁵⁴.

We plotted overall database chemical modifications statistics. Complementary positions from 1 to 19 of cm-siRNAs show comparatively similar frequency distribution of cumulative modifications as depicted in Fig. 3. Overall statistical trend of chemical modifications depicts that terminal positions 1, 2, 3 and 20, 21 are highly altered comparative to central portion as shown in supplementary Fig. S2. This may be probably due to ease of end modifications and their role in nuclease resistance⁵⁵. Further, antisense seems a bit less modified than sense strand, may be due to their differential roles in RISC-machinery⁵⁶. Hence, modifying each strand distinctly may prove a handy tool to improve their cm-siRNA functioning.

Pattern of cumulative occurrence of nucleotides modified on sense and antisense strand of cm-siRNAs is depicted in supplementary Fig. S3. It reveals that majorly one, two or three nucleotides have been engineered for chemical modifications on either siRNA strand. However only in limited studies nucleotides on all twenty-one positions in a siRNA have been modified. Reason for this could be the toxicity associated with increased modifications⁵⁷. Combinations of different unique modifications i.e. how many exclusively different chemical moieties are integrated in cm-siRNA is displayed in supplementary Fig. S4. It elucidates that maximum 5 different combinations of modifications are seen so far in cm-siRNAs. These conglomerates need to be optimized for construction of biologically significant siRNAs²⁴. So, there is further scope to explore these combinations.

For data retrieval and usage we have designed user-friendly tools and web pages. Users can select the unique chemical modification list on web server and check their patterns i.e. on what positions the modifications are reported along with their biological effects. We have provided the browse option with respect to modifications and positions on either sense or antisense strand of siRNA. User can also analyze cm-siRNA sequences based on the positions of modifications. Furthermore, structure search option is also available to draw the structure and search against the database. The database would be updated half yearly/annually or on availability of the enough data in the literature.

Methods

Data acquisition. Literature was exhaustively searched for experimentally validated chemically modified-siRNAs (cm-siRNAs) for extraction of relevant information using keywords related to (i) RNAi (ii) modifications (iii) stability, utilizing advanced search option in PubMed. The search resulted in about 900 articles as on June 2015, which we examined for relevant experimental data on cm-siRNAs. Studies related to chemical modification for their synthesis, lacking complete biological information and reviews were excluded. About 500 potential articles were scrutinized to mine the required fields. Finally from 96 articles, comprehensive information of 4894 cm-siRNAs was extracted.

We have also integrated chemical information about modifications introduced in the siRNAs. This information is derived from public chemical databases namely PubChem⁵⁸, ChemSpider (<http://www.chemspider.com>), ChEMBL (<https://www.ebi.ac.uk/chembl>). Structures of some chemical modifications were hand drawn using MarvinSketch package (<http://www.chemaxon.com>), as they were not available in above chemical repositories.

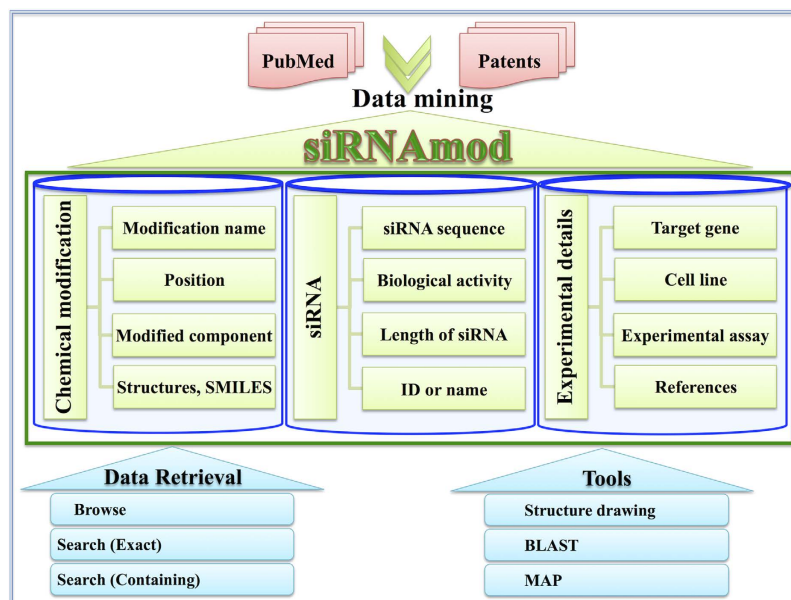


Figure 5. Diagrammatic representation of siRNAmod architecture.

Database organization. siRNAmod provides comprehensive information on experimentally validated cm-siRNA sequences and their chemical modifications. Database is structured to provide information in more than 20 fields broadly divided into; (A) Chemical modification (B) siRNA sequence (C) siRNA activity (D) Experimental details. The database architecture is shown in Fig. 5.

(A) Chemical modification. It includes the information about the chemical moiety and all its related details for both sense and antisense strand distinctly.

(i) Name of chemical modifications for example “2-O-methyl”. If multiple modifications are present then they are separated by asterisk (*) mark e.g. “2,4-Bridged nucleic acid* Deoxythymidine”; (ii) their unique and cumulative numerical magnitude (iii) positions of chemical modifications i.e. positions on the siRNA sequence where the modifications are incorporated. When one modification is present at more than one position, it is represented by the position separated by comma e.g. “1,2,3,4,5” and two modifications at respective positions are separated by asterisks as “1,2,3,4,5 * 23,24”; (iv) modification component of nucleotide presents the information about the modified substructure of the nucleotide i.e. whether sugar, base or phosphate part is modified; (v) structures of modification; (vi) SMILES (simplified molecular input line entry system canonical), InChI (International Chemical Identifier); (vii) systematic name etc.; (viii) natural or non-natural presence of chemical modification.

(B) siRNA sequences. It encompasses information relevant to cm-siRNA sequences and includes (ix) sequence of unmodified siRNA i.e. the basic sequence on which the modification is performed both sense and antisense; (x) length of the siRNA; (xi) Id or name of siRNA used in the respective article. Besides, 435 unmodified siRNAs that were used as controls in the respective studies were also extracted and provided separately in the database.

(C) siRNA activity. It comprises of (xii) biological activity or efficacy of the cm-siRNA as percentage of target mRNA degraded. Besides, inhibition concentration-50 (IC_{50}) values are sometimes provided in the studies instead of percentage inhibition. Qualitative representation of activity is also used to represent the activity of modified siRNA where exact quantitative values were not given, using the terms like high or low activity comparatively.

(D) Experimental details. It covers rest of the relevant information as (xiii) target gene; (xiv) melting temperature of modified siRNA sequence; (xv) siRNA concentration; (xvi) experiment used to check activity; (xvii) cell line or organism; (xviii) transfection method; (xix) post transfection duration; (xx) reference.

Conclusion and Future Developments

Chemical modifications in siRNAs are one of the inevitable steps to ameliorate their therapeutic potential. siRNAmod is the first comprehensive platform of 4894 cm-siRNAs with 128 unique chemical modifications. It provides manually curated information about chemical modification, sequences, siRNA activity and experimental details. Chemical information includes structure, SMILES, InChI to make the database chemically aware. User-friendly web interface is provided to retrieve the desired curated information with ease. Exploration of chemical modifications and analysis in siRNAs and has been accelerated in last decade and data is continuously growing. In future, we would update the database and include cm-siRNA based design tools and algorithm. We hope that siRNAmod would be helpful for further analysis, interpretation and to accelerate the development of siRNA-based therapeutics.

References

1. Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811, doi: 10.1038/35888 (1998).
2. Woessmann, W., Damm-Welk, C., Fuchs, U. & Borkhardt, A. RNA interference: new mechanisms for targeted treatment? *Reviews in clinical and experimental hematology* **7**, 270–291 (2003).
3. Filipowicz, W. RNAi: the nuts and bolts of the RISC machine. *Cell* **122**, 17–20, doi: 10.1016/j.cell.2005.06.023 (2005).
4. Davis, M. E. *et al.* Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **464**, 1067–1070, doi: 10.1038/nature08956 (2010).
5. Joo, M. K., Yhee, J. Y., Kim, S. H. & Kim, K. The potential and advances in RNAi therapy: Chemical and structural modifications of siRNA molecules and use of biocompatible nanocarriers. *Journal of controlled release : official journal of the Controlled Release Society*, doi: 10.1016/j.jconrel.2014.05.030 (2014).
6. Gallas, A., Alexander, C., Davies, M. C., Puri, S. & Allen, S. Chemistry and formulations for siRNA therapeutics. *Chemical Society reviews* **42**, 7983–7997, doi: 10.1039/c3cs35520a (2013).
7. Gaglione, M. & Messere, A. Recent progress in chemically modified siRNAs. *Mini reviews in medicinal chemistry* **10**, 578–595 (2010).
8. Stephenson, M. L. & Zamecnik, P. C. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proceedings of the National Academy of Sciences of the United States of America* **75**, 285–288 (1978).
9. Wilson, C. & Keefe, A. D. Building oligonucleotide therapeutics using non-natural chemistries. *Current opinion in chemical biology* **10**, 607–614, doi: 10.1016/j.cbpa.2006.10.001 (2006).
10. Elmen, J. *et al.* Locked nucleic acid (LNA) mediated improvements in siRNA stability and functionality. *Nucleic acids research* **33**, 439–447, doi: 10.1093/nar/gki193 (2005).
11. Kanski, D. M. *et al.* Analysis of acyclic nucleoside modifications in siRNAs finds sensitivity at position 1 that is restored by 5'-terminal phosphorylation both *in vitro* and *in vivo*. *Nucleic acids research* **38**, 660–671, doi: 10.1093/nar/gkp913 (2010).
12. Takahashi, M. *et al.* Intracellular stability of 2'-O-Me-4'-thioribonucleoside modified siRNA leads to long-term RNAi effect. *Nucleic acids research* **40**, 5787–5793, doi: 10.1093/nar/gks204 (2012).
13. Prakash, T. P. *et al.* Positional effect of chemical modifications on short interference RNA activity in mammalian cells. *Journal of medicinal chemistry* **48**, 4247–4253, doi: 10.1021/jm050044o (2005).
14. Soutschek, J. *et al.* Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* **432**, 173–178, doi: 10.1038/nature03121 (2004).
15. Petersen, M. & Wengel, J. LNA: a versatile tool for therapeutics and genomics. *Trends in biotechnology* **21**, 74–81, doi: 10.1016/S0167-7799(02)00038-0 (2003).
16. Grunweller, A. *et al.* Comparison of different antisense strategies in mammalian cells using locked nucleic acids, 2'-O-methyl RNA, phosphorothioates and small interfering RNA. *Nucleic acids research* **31**, 3185–3193 (2003).
17. Glud, S. Z. *et al.* Naked siLNA-mediated gene silencing of lung bronchoepithelium EGFP expression after intravenous administration. *Oligonucleotides* **19**, 163–168, doi: 10.1089/oli.2008.0175 (2009).
18. Langkjaer, N., Pasternak, A. & Wengel, J. UNA (unlocked nucleic acid): a flexible RNA mimic that allows engineering of nucleic acid duplex stability. *Bioorganic & medicinal chemistry* **17**, 5420–5425, doi: 10.1016/j.bmc.2009.06.045 (2009).
19. Aboul-Fadl, T. Antisense oligonucleotides: the state of the art. *Current medicinal chemistry* **12**, 2193–2214 (2005).
20. Detzer, A. & Sczakiel, G. Phosphorothioate-stimulated uptake of siRNA by mammalian cells: a novel route for delivery. *Current topics in medicinal chemistry* **9**, 1109–1116 (2009).
21. Chiu, Y. L. & Rana, T. M. siRNA function in RNAi: a chemical modification analysis. *Rna* **9**, 1034–1048 (2003).
22. Layzer, J. M. *et al.* *In vivo* activity of nuclease-resistant siRNAs. *Rna* **10**, 766–771 (2004).
23. Odadzic, D. *et al.* Synthesis of 2'-O-modified adenosine building blocks and application for RNA interference. *Bioorganic & medicinal chemistry* **16**, 518–529, doi: 10.1016/j.bmc.2007.09.019 (2008).
24. Bramsen, J. B. *et al.* A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity. *Nucleic acids research* **37**, 2867–2881, doi: 10.1093/nar/gkp106 (2009).
25. Somoza, A., Silverman, A. P., Miller, R. M., Chelliserrykattil, J. & Kool, E. T. Steric effects in RNA interference: probing the influence of nucleobase size and shape. *Chemistry* **14**, 7978–7987, doi: 10.1002/chem.200800837 (2008).
26. Sipa, K. *et al.* Effect of base modifications on structure, thermodynamic stability, and gene silencing activity of short interfering RNA. *Rna* **13**, 1301–1316, doi: 10.1261/rna.538907 (2007).
27. Peacock, H., Kannan, A., Beal, P. A. & Burrows, C. J. Chemical modification of siRNA bases to probe and enhance RNA interference. *The Journal of organic chemistry* **76**, 7295–7300, doi: 10.1021/jo2012225 (2011).
28. Hall, A. H., Wan, J., Shaughnessy, E. E., Ramsay Shaw, B. & Alexander, K. A. RNA interference using boranophosphate siRNAs: structure-activity relationships. *Nucleic acids research* **32**, 5991–6000, doi: 10.1093/nar/gkh936 (2004).
29. Potenza, N. *et al.* RNA interference in mammalia cells by RNA-3'-PNA chimeras. *International journal of molecular sciences* **9**, 299–315 (2008).
30. Detzer, A., Overhoff, M., Mescalchin, A., Rompf, M. & Sczakiel, G. Phosphorothioate-stimulated cellular uptake of siRNA: a cell culture model for mechanistic studies. *Current pharmaceutical design* **14**, 3666–3673 (2008).
31. Braasch, D. A. *et al.* Biodistribution of phosphodiester and phosphorothioate siRNA. *Bioorganic & medicinal chemistry letters* **14**, 1139–1143, doi: 10.1016/j.bmcl.2003.12.074 (2004).
32. Butora, G. *et al.* Nucleoside optimization for RNAi: a high-throughput platform. *Journal of the American Chemical Society* **133**, 16766–16769, doi: 10.1021/ja2068774 (2011).
33. Broering, R. *et al.* Chemical modifications on siRNAs avoid Toll-like-receptor-mediated activation of the hepatic immune system *in vivo* and *in vitro*. *International immunology* **26**, 35–46, doi: 10.1093/intimm/dxt040 (2014).
34. Ren, Y. *et al.* siRecords: a database of mammalian RNAi experiments and efficacies. *Nucleic acids research* **37**, D146–149, doi: 10.1093/nar/gkn817 (2009).
35. Chalk, A. M., Warfinge, R. E., Georgii-Hemming, P. & Sonnhammer, E. L. siRNAdb: a database of siRNA sequences. *Nucleic acids research* **33**, D131–134, doi: 10.1093/nar/gki136 (2005).
36. Truss, M. *et al.* HuSiDa—the human siRNA database: an open-access database for published functional siRNA sequences and technical details of efficient transfer into recipient cells. *Nucleic acids research* **33**, D108–111, doi: 10.1093/nar/gki131 (2005).
37. Thakur, N., Qureshi, A. & Kumar, M. VIRsiRNAdb: a curated database of experimentally validated viral siRNA/shRNA. *Nucleic acids research* **40**, D230–236, doi: 10.1093/nar/gkr1147 (2012).
38. Tyagi, A. *et al.* HIVsirDB: a database of HIV inhibiting siRNAs. *PloS one* **6**, e25917, doi: 10.1371/journal.pone.0025917 (2011).
39. Cantara, W. A. *et al.* The RNA Modification Database, RNAMDB: 2011 update. *Nucleic acids research* **39**, D195–201, doi: 10.1093/nar/gkq1028 (2011).
40. Herraiz, A. Biomolecules in the computer: Jmol to the rescue. *Biochemistry and molecular biology education : a bimonthly publication of the International Union of Biochemistry and Molecular Biology* **34**, 255–261, doi: 10.1002/bmb.2006.494034042644 (2006).
41. Ozcan, G., Ozpolat, B., Coleman, R. L., Sood, A. K. & Lopez-Berestein, G. Preclinical and clinical development of siRNA-based therapeutics. *Advanced drug delivery reviews*, doi: 10.1016/j.addr.2015.01.007 (2015).
42. Burnett, J. C., Rossi, J. J. & Tiemann, K. Current progress of siRNA/shRNA therapeutics in clinical trials. *Biotechnology journal* **6**, 1130–1146, doi: 10.1002/biot.201100054 (2011).

43. Lares, M. R., Rossi, J. J. & Ouellet, D. L. RNAi and small interfering RNAs in human disease therapeutic applications. *Trends in biotechnology* **28**, 570–579, doi: 10.1016/j.tibtech.2010.07.009 (2010).
44. Engels, J. W. Gene silencing by chemically modified siRNAs. *New biotechnology* **30**, 302–307, doi: 10.1016/j.nbt.2012.07.002 (2013).
45. Turner, J. J., Jones, S. W., Moschos, S. A., Lindsay, M. A. & Gait, M. J. MALDI-TOF mass spectral analysis of siRNA degradation in serum confirms an RNase A-like activity. *Molecular bioSystems* **3**, 43–50, doi: 10.1039/b611612d (2007).
46. Bramsen, J. B. & Kjems, J. Development of Therapeutic-Grade Small Interfering RNAs by Chemical Engineering. *Frontiers in genetics* **3**, 154, doi: 10.3389/fgene.2012.00154 (2012).
47. Foster, D. J. *et al.* Comprehensive evaluation of canonical versus Dicer-substrate siRNA *in vitro* and *in vivo*. *Rna* **18**, 557–568, doi: 10.1261/rna.031120.111 (2012).
48. Nallagatla, S. R. & Bevilacqua, P. C. Nucleoside modifications modulate activation of the protein kinase PKR in an RNA structure-specific manner. *Rna* **14**, 1201–1213, doi: 10.1261/rna.1007408 (2008).
49. Haringsma, H. J. *et al.* mRNA knockdown by single strand RNA is improved by chemical modifications. *Nucleic acids research* **40**, 4125–4136, doi: 10.1093/nar/gkr1301 (2012).
50. Collingwood, M. A. *et al.* Chemical modification patterns compatible with high potency dicer-substrate small interfering RNAs. *Oligonucleotides* **18**, 187–200, doi: 10.1089/oli.2008.0123 (2008).
51. Somoza, A., Terrazas, M. & Eritja, R. Modified siRNAs for the study of the PAZ domain. *Chemical communications* **46**, 4270–4272, doi: 10.1039/c003221b (2010).
52. Peel, B. J., Hagen, G., Krishnamurthy, K. & Desaulniers, J. P. Conjugation and Evaluation of Small Hydrophobic Molecules to Triazole-Linked siRNAs. *ACS medicinal chemistry letters* **6**, 117–122, doi: 10.1021/ml500260j (2015).
53. Kenski, D. M., Willingham, A. T., Haringsma, H. J., Li, J. J. & Flanagan, W. M. *In vivo* activity and duration of short interfering RNAs containing a synthetic 5'-phosphate. *Nucleic acid therapeutics* **22**, 90–95, doi: 10.1089/nat.2011.0333 (2012).
54. Kamiya, Y. *et al.* Enhancement of stability and activity of siRNA by terminal substitution with serinol nucleic acid (SNA). *Chembiochem: a European journal of chemical biology* **15**, 2549–2555, doi: 10.1002/cbic.201402369 (2014).
55. Huang, Y. *et al.* Exploring directional invasion of serum nuclease into siRNA duplexes by asymmetrical terminal modifications. *ChemMedChem* **9**, 2111–2119, doi: 10.1002/cmcd.201402115 (2014).
56. Hamada, M. *et al.* Effects on RNA interference in gene expression (RNAi) in cultured mammalian cells of mismatches and the introduction of chemical modifications at the 3'-ends of siRNAs. *Antisense & nucleic acid drug development* **12**, 301–309, doi: 10.1089/108729002761381285 (2002).
57. Amarzguioui, M., Holen, T., Babaie, E. & Prydz, H. Tolerance for mutations and chemical modifications in a siRNA. *Nucleic acids research* **31**, 589–595 (2003).
58. Wang, Y. *et al.* PubChem BioAssay: 2014 update. *Nucleic acids research* **42**, D1075–1082, doi: 10.1093/nar/gkt978 (2014).

Acknowledgements

Financial support for this work is provided by CSIR-Institute of Microbial Technology (OLP 0083), Council of Scientific and Industrial Research (BSC0121) and Department of Biotechnology, Government of India (GAP0001). Funding for open access charge: CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh, India.

Author Contributions

M.K. conceived the idea, planned the design, visualization and tools pipeline and coordinated the entire project. S.D. and A.T. manually collected and curated all the data. S.D. and A.Q. designed the web server part of the database. S.D. and M.K. performed data analysis, interpreted results and drafted the manuscript. All authors read and approved the final manuscript.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Dar, S. A. *et al.* siRNAmoD: A database of experimentally validated chemically modified siRNAs. *Sci. Rep.* **6**, 20031; doi: 10.1038/srep20031 (2016).



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