ChiTaRS 5.0: the comprehensive database of chimeric transcripts matched with druggable fusions and 3D chromatin maps

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ABSTRACT

Chimeric RNA transcripts are formed when exons from two genes fuse together, often due to chromosomal translocations, transcriptional errors or trans-splicing effect. While these chimeric RNAs produce functional proteins only in certain cases, they play a significant role in disease phenotyping and progression. ChiTaRS 5.0 (http://chitars.md.biu.ac.il/) is the latest and most comprehensive chimeric transcript repository, with 111 582 annotated entries from eight species, including 23 167 known human cancer breakpoints. The database includes unique information correlating chimeric breakpoints with 3D chromatin contact maps, generated from public datasets of chromosome conformation capture techniques (Hi–C). In this update, we have added curated information on druggable fusion targets matched with chimeric breakpoints, which are applicable to precision medicine in cancers. The introduction of a new section that lists chimeric RNAs in various cell-lines is another salient feature. Finally, using text-mining techniques, novel chimeras in Alzheimer’s disease, schizophrenia, dyslexia and other diseases were collected in ChiTaRS. Thus, this improved version is an extensive catalogue of chimeras from multiple species. It extends our understanding of the evolution of chimeric transcripts in eukaryotes and contributes to the analysis of 3D genome conformational changes and the functional role of chimeras in the etiopathogenesis of cancers and other complex diseases.

INTRODUCTION

The transcriptome in eukaryotes is composed of single-stranded sequences of RNAs transcribed from various locations in the total genome. Although most RNA transcripts can be traced back to a single locus, exons from two different genes or from two copies of the same gene sometimes fuse together, leading to the formation of chimeric RNAs (1–22). The various causes of such fusions include transcriptional errors, trans-splicing effects and chromosomal translocations (6,14). Thus, two unrelated genomic loci on different chromosomes may produce a chimeric transcript through a genomic rearrangement event or due to trans-splicing. Similarly, a read-through transcript of two adjacent genomic loci may produce chimeric RNAs (8–9,21). The possibility is high that chimeras are artefacts of template switching by the reverse transcriptase enzyme (considering the difficulty of performing reverse transcriptase-free assays) (9,16). Nonetheless, several studies have identified chimeric transcripts that have also been translated into proteins. This establishes their authenticity and further motivates scientists to curate a list of known chimeras (19–26). A direct correlation has been suggested between the presence of chimeras in the genome and their role in tumorigenesis (18,19). The transcriptome becomes extremely more complex in the context of cancer, due to a high proportion of genomic rearrangements, nucleotide polymorphisms and alterations of the splicing machinery.

One of the most renown gene fusions identified in solid tumors is the TMPRSS2-ERG chimera, a well-documented biomarker of prostate cancer. This chimera has been identified in a high frequency of tested patient samples (28). Novel means, like the delivery of specific siRNAs by targeted liposomal nanovectors and the use of peptidomimetic inhibitors have been attempted, with the aim of mitigating the spread of prostate cancer (29,30). Similarly, FGFR3-TACC3 fusions have been identified as driving factors for cancer progression in multiple tissue types like bladder, lung and brain.
These fusions are currently being targeted using the drug erdafitinib (31). Such cases highlight the need to consolidate all known chimeras related to cancer and other disorders, together with information about their breakpoints. This will enable their use as diagnostic tools and as potential drug targets. Gene fusion associations in both solid and liquid tumour development, as well as in other genetic disorders, have been confirmed by the detection of chimeras in cell-lines, using short read sequencing strategies (20). Chimeric transcripts have been shown to be significantly more tissue specific by nature than non-chimeric transcripts (16). Additionally, chimeric RNAs appear not to be a distinct feature of tumour physiology. Recurrent gene fusions have been identified in non-cancerous tissues, as well as in normal cells (27). Chimeras have also been found to lose some of their functional domains, and therefore actively compete with their wild-type genes. This leads to a dominant-negative effect in cancers and other diseases (17). Recent studies have intercepted contact maps of chromosome conformation capture techniques (Hi-C) with known chromosomal translocations. A marked increase has been identified in the correlation between certain tissue types and the Hi-C contact frequencies (32).

As next generation sequencing (NGS) has become the norm for genomic studies in recent years, the identification of chimeric transcripts has increased at an exponential rate (33). Several efforts have been made to catalogue the chimeric transcripts from literature, using a variety of computational methods and text-mining techniques (34). These have resulted in the creation of databases such as the Mitelman database, ChimerDB 3.0, COSMIC, dbCRID, HybridDB, TICdb and FusionGDB. (23,33,35–40). These databases have actively supported research in their respective domains. However, ChiTaRS stands out as a unique server that integrates EST and mRNA sequences, and literature resources, with RNA-sequencing data, expression level and tissue specificity of chimeric transcripts in various tissues and organisms.

The evolution of ChiTaRS

The ChiTaRS database was originally designed to incorporate chimeric transcripts from three organisms (human, mouse and fruit fly) (41). Subsequently, from ChiTaRS-2.1, the database was extended to eight organisms. This increased the evidence of chimeric transcript conservation across these species (42,43). The database was created using sustained bioinformatics analyses of the expressed sequence tags (ESTs) and mRNA sequences from GenBank (44). We also extended our resource of published chimeras, with RNA expression level as verified by RNA sequencing, and whose translated proteins have been shown by means of shotgun and targeted mass-spectrometry analyses (16,42). Interactive links to the protein-protein interaction maps of all the chimeric proteins have also been incorporated into the ChiTaRS database, with the help of the ChiPPI tool, introduced in ChiTaRS-3.1 (43,45). Moreover, ChiTaRS enables visualization of the transcripts and their junction sites by SpliceGrapher, using the genome annotation for the respective organisms from the UCSC genome browser (46). Additionally, an exclusive feature of the ChiTaRS database is the assessment of junction consistency. This enables ranking chimeras according to the evidence of consistent junction sites. This feature, introduced in ChiTaRS-2.1, has proven beneficial to researchers seeking experimental confirmation of highly ranked chimeric transcripts (42). The ChiTaRS-4.1 version of the database, which was released online in 2018, announced the addition of Hi–C contact maps in nearly 5600 chimeras. This marked an important adjunct to the database. The most recent release, ChiTaRS 5.0, consists of a novel section on druggable fusions, mined from various sources of literature. The database also introduces a distinct section for chimeras identified in 531 human cell-line systems. Moreover, using our ProtFus method (34), we added novel chimeras from neurodegenerative and autoimmune diseases like Alzheimer’s disease, schizophrenia and rheumatoid arthritis, thereby empowering research in these other fields. Such features, coupled with many more salient existing database functionalities, make ChiTaRS one of the most up-to-date and sought-after resources for chimeric transcripts.

Improvements in ChiTaRS

The significant updates and improvements to the ChiTaRS database since the last published edition are tabulated in Table 1. The major improvements include: the introduction of a segment of druggable fusion targets matched with chimeric breakpoints, the addition of 3D chromatin contact maps from publicly available Hi–C datasets in four organisms, the creation of a discrete section of chimeric transcripts identified in various human and mice cell-lines, and extension of the annotation of detected chimeras to UniProt, NCBI, GeneCards, PubMed and other public resources.

Database content enhancement

For the current 2019 release of the database, 111 582 chimeric RNAs were collated from 1 393 046 EST and mRNA sequences of eight species (namely, Homo sapiens, Mus musculus, Drosophila melanogaster, Rattus norvegicus, Bos Taurus, Saccharomyces cerevisiae, Sus scrofa and Danio rerio) retrieved from NCBI/GenBank (44). All chimeric transcripts were aligned to their respective reference genome assemblies using the UCSC BLAT program (47). The details of the reference genomes used for each species are provided in Supplementary Table S1. We included a chimera in the database if its first and second sequence tracts had a minimum identity of 95% and a minimum length of 50nt; and if these two sequences could not be mapped linearly to the reference genome. The number of chimeras detected in each organism is presented in Table 1. Moreover, 23 167 unique cancer breakpoints were collected from the recent publications by Mertens et al. and from the Mitelman collection, together comprising ~11 000 more breakpoints than previously collated (33,35). Thus, we verified, manually, 11 650 breakpoints to confirm their accuracy, with the help of >7700 PubMed articles. Notably, haematological tumour malignancies correlated with a high frequency of reported fusions, particularly, acute lymphoblastic leukaemia (ALL, 710 chimeras), acute myeloid
Table 1. Improvements in ChiTaRS 5.0: significant enhancement over previous database editions

| Content                                      | ChiTaRS | ChiTaRS 2.1 | ChiTaRS 3.1 | ChiTaRS 5.0 | Relevance                                                                 |
|----------------------------------------------|---------|-------------|-------------|-------------|---------------------------------------------------------------------------|
| The collection of chimeric transcripts       | 16282 (total), 9379 (Homo sapiens), 4828 (Mus musculus), 2055 (D. melanogaster) | 29164 (total), 20 753 (Homo sapiens), 6226 (Mus musculus), 2151 (D. melanogaster), 4 (Bos taurus), 8 (Rattus norvegicus), 4 (Danio rerio), 5 (S. cerevisiae), 13 (Sus scrofa) | 34 922 (total), 24 608 (Homo sapiens), 7457 (Mus musculus), 2740 (D. melanogaster), 6 (Bos taurus), 10 (Rattus norvegicus), 7 (Danio rerio), 5 (S. cerevisiae), 89 (Sus scrofa) | 111 582 (total), 66 243 (Homo sapiens), 41 584 (Mus musculus), 3052 (D. melanogaster), 19 (Bos taurus), 20 (Rattus norvegicus), 67 (Danio rerio), 305 (S. cerevisiae), 292 (Sus scrofa) | Extension of the collection for all eight organisms by ∼76 600 new entries |
| Disease Breakpoints                          | 1280    | 1428        | 11 714      | 23 167      | Extension by >11 000 new entries to include unique fusion transcripts with breakpoint information associated with cancer and other complex diseases |
| Manually verified breakpoints                | 456     | 1428        | 10 285      | 680         | Additional breakpoints verified manually                                    |
| Druggable fusions                            | No      | No          | No          | No          | A novel feature providing information on the use of the chimeric breakpoint as a drug target |
| Hi-C (3D Chromatin Contact Map) chimeras     | No      | No          | No          | 5597        | The addition of ∼5600 chimeras, now matched with 3D chromatin contact maps from Hi–C data |
| Chimeric Protein-Protein interaction (ChiPPI) networks | No      | No          | 2081 (validated), 22527 (predicted) | 9973 (validated), 42 058 (predicted) | Additional protein-protein network analysis maps for ∼27 000 chimeras |
| RNA-seq verified chimeras                    | 175     | 337         | 435         | 937         | Extension of the number of chimeras verified in RNA-seq experiments by ∼500 |
| Sense-antisense chimeras                     | No      | 6044        | 6485        | 7521        | The incorporation of ∼1000 additional sense-antisense chimeras |
| Chimeras identified in cell-lines            | No      | No          | No          | 2411        | The identification of ∼2400 chimeras across 531 human cell lines |
| Links to external data repositories (GeneCards, OMIM, Pubmed, Ensembl, SNOMED CT & RefSeq) | No      | No          | 33 124      | 136 458     | The addition of ∼100 000 links to multiple data repositories for ease of correlation |

leukaemia (AML, 639 chimeras) and acute myelomonocytic leukaemia (AMMoL, 599 chimeras) topped the list. Further to the focus on chimeras in cancer, we added novel chimeras that have been linked to the progression of other neurodegenerative and autoimmune disorders, such as dyslexia, schizophrenia, rheumatoid arthritis and systemic lupus erythematosus. To facilitate the understanding of the biological significance of each chimera, we updated the GenBank, RefSeq and Mitelman cross-references for all the genes (total of 202 460 links) (44,48). UniProt, Ensembl, FlyBase (for D. melanogaster), SGD (for S. cerevisiae), GeneCard and OMIM links (∼256 000 links) for the parental genes are also supported in the current version of the ChiTaRS database. This yields a valuable resource for conducting in-depth analyses of chimeric transcripts (49–54). Detailed information regarding available resources for each organism is provided in Supplementary Table S2. We have also added a new section specifically for 2411 gene fusions from 531 cell lines of human and mouse origin. The cell lines with the highest frequency of chimeric transcripts expressed include HCC1263 (human colorectal adenocarcinoma), VCaP (prostate cancer) and MDA-MB-361 (breast cancer derived from metastatic site – brain), containing 25, 22 and 21 chimeras, respectively.

Interface upgrades in ChiTaRS 5.0

The user interface of ChiTaRS 5.0 has also been upgraded to provide the end-user with a seamless user experience, combining the latest database functionalities with the database existing treasure trove of options. We have added a check-box option in the ‘Full Collection and Search’ tab for the exclusive search of chimeras with Hi–C contacts (Figure 1). In ChiTaRS-3.1, we introduced protein-protein network
Figure 1. The ‘Full Collection & Search’ tab on the updated ChiTaRS 5.0 database (http://chitars.md.biu.ac.il/bin/search.pl). The new interface provides options to perform custom searches for chimeras with matched Hi–C contact maps, RNAseq evidences, etc. A sample search is shown below, with each chimera result containing links to external data repositories like NCBI, UniProt, SpliceGrapher, PubMed, GeneCards and ChiPPI. This provides additional information at the user’s disposal.
analysis charts for each chimeric protein from chimeric Protein-Protein interactions (ChiPPI) (45). This enabled the end-user to find ‘missing’ and ‘known’ interactors for the fusion protein, based on the preserved domains from the parental genes (45). We have now added additional pop-up windows for these ChiPPI networks, and extended annotations of genes and links to PubMed references. A separate tab now facilitates searching for druggable fusions and breakpoints (Supplementary Figure S1). A tab exclusively for chimeras identified in cell lines has also been introduced (Supplementary Figure S2). These features simplify and accelerate analyses, with reduced turnaround time for the end-users. A new user submission form offers users the option to submit chimeric transcripts they have identified. These will be added to the database after due verification. The process for submission is explained in Supplementary Figure S3.

**Druggable fusions as tailored therapy targets**

Over the years, several papers have documented the role of gene fusions as drivers of cancer and tumour progression. Even rare chimeric transcripts, occurring in low frequencies, can be potential functional drivers (55). In certain cancer types, some fusions recur frequently, generally during tumour initiation (56). Prior to the NGS revolution, identifying gene fusions was extremely difficult. However, currently available NGS technology offers great promise in the field of precision medicine, particularly for using chimeric proteins as druggable fusion targets. Gene fusions can be highly specified. The best examples in support of this theory are the BCR-ABL1 fusion in chronic myeloid leukaemia (CML) and the EML4-ALK gene fusion in non-small cell lung carcinoma (NSCLC). The BCR-ABL1 chimera is used as a target for tyrosine kinase inhibitors like imatinib and nilotinib (57). Drugs like crizotinib and alectinib, are used to treat NSCLC by targeting the EML4-ALK fusion event (58). The ALK inhibitor crizotinib is approved for treatment of patients with NSCLC in Europe, Japan, the United States and other countries. On the other hand, alectinib is a more selective oral ALK inhibitor, and several research groups have demonstrated its effectiveness in systemic disease and in the central nervous system (59). In this version of ChiTaRS, we present a new manually-verified collection of druggable chimeras for various malignancies, linked with their breakpoint information, Medical Subject Headings (MeSH), the Diseases Database (DiseasesDB) and PubMed links to the published manuscripts. We identified 37 drugs/drug targets, including imatinib, crizotinib and sorafenib. These chimeric protein-based therapeutics were curated from us systematic data mining of published literature and open source repositories like DEPO (56,60). A list of commonly used drugs that target fusions is provided in Table 2. We have provided as separate files the FASTA sequences, the chimeric junction sequence and the drugs that are currently used to target these druggable chimeras. These can be accessed from the ‘Downloads’ tab on the ChiTaRS website (http://chitars.md.biu.ac.il/downloads.html). Users may consider this as a ‘gold-standard’ druggable fusion dataset to standardize their methods for fusion prediction and testing. The complete list of such drugs can be retrieved from the ChiTaRS database online. We have provided a searchable list of drugs/chimeras and other features, together with the relevant scientific information (Supplementary Figure S1).

**Hi–C contact map correlations with chimeric junctions**

The chromosomal translocations frequently occurring in cancer and other disorders often contribute substantially to the progression of these disorder as expressed drivers. Translocations from multiple tissue types have been shown to display high rates of Hi–C contact frequencies. This shapes the 3D genome architecture of the species and can be used in detecting/controlling these abnormalities (32,61–62). Thus, the increased frequency of these specific translocations, together with the existence of rearrangement hotspots, attest to the role of certain internal cellular mechanisms that promulgate specific regions to cross-link. The development of Hi–C, an NGS-based protocol enables describing contact probabilities across the human genome (63). Originally designed to detect known and novel gene fusions in cell-lines, Hi–C has transformed into a robust protocol that can be applied to tissue samples from humans and other organisms, and even detect copy-number variations (64). The ChiTaRS 5.0 database includes Hi–C chomat contact maps from public datasets for four organisms, namely, human, mouse, fruit fly and yeast. We have included nearly 5600 chimeras, spanning across 14 cell lines and tissue samples. In humans, we included chimeras with 3D chromatin contacts in K562, HMEC, imr90, gm12878, NHEK, KBM-7, HUVEC and HeLa cell-lines. We also added Hi–C contacts for chimeras detected in Patski, EKLF−/−, F123 and CH12-LX cell-lines from mice. Detailed information about the public datasets used can be retrieved from the ‘Methods’ tab of the ChiTaRS website. To date, the mechanisms by which chromatin changes are produced in diseased conditions and their epigenetics are poorly understood. Hi–C contributes to this knowledge as it is a cost-effective method, with high efficiency of identifying these translocations, even when signals are low (64). This is because the contacts generally tend to be on either side of the breakpoint and, therefore, fall into the cis and not the trans region (65). Such chimeric transcripts, with matched 3D chromatin maps, may also serve as suitable diagnostic / prognostic biomarkers of disease progression (66). During our process of curating chimeras with Hi–C contacts, we noticed a marked correlation in the ratio of translocations to sense-antisense chimeras that we had described previously (42), in relation to 3D chromatin contact maps. Therefore, we propose that changes in chromatin topology influences the formation of sense-antisense chimeras. We also noticed the presence of such chimeras with the conserved Hi–C contacts in certain genes like CDH2, APP and TTN. These are conserved across species, among humans, mice and fruit flies.

We examined the presence of chimeras in evolutionarily conserved genes across three species (H. sapiens, M. musculus and D. melanogaster) and identified 70 chimeras, of which 29 were overrepresented for three genes only, namely APP, CTBP1 and EEFF2 (human) (Figure 2). The 29 chimeras from these three genes are listed in Table 3. The complete list of 70 chimeras is provided in Supplementary
Figure 2. A phylogenetic tree representing the evolution between human (H), mouse (M) and fruit-fly (F) chimeras (represented by NCBI id), with matched Hi–C contacts in APP, CTBP1 and EEF2 genes. We found evolutionary conservation in the Hi–C contacts between orthologs for different organisms and paralogs for the same organisms.
Table 2. List of chimeric RNAs frequently being used as drug targets

| S.No. | Drug       | Gene fusion          | References (PubMed ID)       |
|-------|------------|----------------------|------------------------------|
| 1     | Alectinib  | EML4-ALK             | 25228534, 28890946           |
| 2     | ATRA + ATO | PML-RARA             | 15103387                     |
| 3     | AZD4547    | FGFR1-TACC1          | 28890946                     |
| 4     | Crizotinib | RANBP2-ALK           | 20979472, 28890946           |
| 5     | Dasatinib  | EML4-ALK             | 27748748, 28890946           |
|       |            | PTPRZ1-MET           |                              |
| 6     | Entrectinib| TPM3-NTRK1           | 21863287, 21562040           |
|       |            | ETV6-NTRK3           | 24705251, 28890946           |
|       |            | VCL-NTRK2            |                              |
|       |            | AGBL4-NTRK2          |                              |
| 7     | Erdafinitib| FGFR3-TACC3          | 28890946                     |
| 8     | Foretinib  | PTPRZ1-MET           | 27748748                     |
| 9     | Imatinib   | COL1A1-PDGFB         |                              |
|       |            | BCR-PDGFB            |                              |
|       |            | EM1-ABL1             |                              |
|       |            | NUP214-ABL1          |                              |
|       |            | COL1A1-PDGFB         |                              |
|       |            | BCR-ABL1             |                              |
| 10    | Lapatinib  | TENM4-NRG1           | 24727320                     |
| 11    | Larotrectinib| TPM3-NTRK1         | 28890946, 29606586           |
| 12    | Nilotinib  | BCR-ABL1             | 21562040, 28890946           |
| 13    | Ponatinib  | FGFR1-TACC1          | 29617662                     |
| 14    | Sorafenib  | SLC45A3-BRAF         | 20526349, 24727320           |
| 15    | Trastuzumab| ERBB2-CLDN7          | 28890946                     |

Table S3. Amyloid precursor protein (APP) gene codes for a protein of the same name and is found in many tissues and organs of the body, especially the brain and the spinal cord. While the exact function of the APP gene is yet to be identified, recent studies have shown its involvement in the migration of nerve cells in the brain during early development. Mutation or dysregulation of the APP gene has also been shown in a high proportion of individuals with Alzheimer’s disease, and in hereditary cerebral amyloid angiopathy (67). C-Terminal Binding Protein 1 (CTBP1) is a phosphoprotein and a known transcriptional repressor, and dimerizes with CTBP2, its closely related gene. Alterations in CTBP1 are known to cause developmental delay, Wolf-Hirschhorn syndrome and other conditions (68). Eukaryotic translation elongation factor (EEF2) is an essential component of the protein synthesis pathway and is involved in GTP-dependent translocation of nascent protein chains (69). EEF2 has been found to be dysregulated in tracheal carcinoma, spinocerebellar ataxia and other disorders. We identified 10 chimeras across the three species in the conserved APP gene (App in the mouse and AppI in the fruit fly), five chimeras in the CTBP1 gene (Ctbp2 in the mouse, CtBP in the fruit fly) and 14 chimeras in the EEF2 gene (mouse orthologue Eef2, fruit fly orthologue EF2). We analysed the FASTA sequence to identify the presence of any functional domains that have been evolutionarily conserved. Data of the multiple sequence alignment and phylogenetic separation are presented in Figure 2.

CONCLUSIONS AND PERSPECTIVES

The comprehensive ChiTaRS 5.0 database will serve as a critical tool dedicated to the study of chimeric transcripts in eukaryotes at ‘multi’-omics levels, spanning genomic, transcriptomic and proteomic domains. Our resources such as ChiTaRS, ProtFus and ChiPPI (34,41–43,45) are repeatedly referenced as valuable resources for fusion discovery and annotation (70). The updated version 5.0 of the ChiTaRS database provides a phenomenal increase in annotated and verified chimeric transcripts, compared to the previous ChiTaRS releases, and includes a significant extension of specific research-oriented features like druggable fusions and 3D chromatin contact maps. ChiTaRS 5.0 provides extensive experimental evidence for chimeras specific to cancers, and auto-immune (rheumatoid arthritis, systemic lupus erythematosus) and neurodegenerative disorders (such as Alzheimer’s and Parkinson’s diseases). These can be effectively applied in the planning of new experiments or for the analysis of large-scale sequencing experiments. Ongoing international consortia like ICGC, PanCancer, 100 000 Genome Project, ADNI, NIAGADS and gnomAD will benefit from this database and from all incremental additions to it, for improving the process of chimera identification and validation. To conclude, the updated ChiTaRS database is designed to advance the field of cancer research as well as evolutionary biology, and ultimately contribute to the therapeutic stratification of diseases using chimeric transcripts, thus validated as potential biomarkers.

DATA AVAILABILITY

The ChiTaRS 5.0 content will be continuously maintained and updated every six months. The database is now publicly accessible at http://chitars.md.biu.ac.il. The previous versions 3.1 and 2.1 are accessible at http://biodb.md.biu.
SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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