SMDB: pivotal somatic sequence alterations reprogramming regulatory cascades

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ABSTRACT

Binding motifs for transcription factors, RNA-binding proteins, microRNAs (miRNAs), etc. are vital for proper gene transcription and translation regulation. Sequence alteration mechanisms including single nucleotide mutations, insertion, deletion, RNA editing and single nucleotide polymorphism can lead to gains and losses of binding motifs; such consequentially emerged or vanished binding motifs are termed ‘somatic motifs’ by us. Somatic motifs have been studied sporadically but have never been curated into a comprehensive resource. By analyzing various types of sequence altering data from large consortia, we successfully identified millions of somatic motifs, including those for important transcription factors, RNA-binding proteins, miRNA seeds and miRNA–mRNA 3′-UTR target motifs. While a few of these somatic motifs have been well studied, our results contain many novel somatic motifs that occur at high frequency and are thus likely to cause important biological repercussions. Genes targeted by these altered motifs are excellent candidates for further mechanism studies. Here, we present the first database that hosts millions of somatic motifs ascribed to a variety of sequence alteration mechanisms.

INTRODUCTION

Somatic mutations occurring in life-supporting protein-encoding genes can cause severe adverse effects on human health (1). Non-coding somatic mutations can also have dreadful consequences (2). Non-coding somatic mutations become especially damaging if they alter cis-elements for regulator molecules, including transcription factors (TFs) (3), RNA-binding proteins (RBPs) (4), microRNA (miRNA) seeds (5), miRNA–mRNA 3′-UTR targeting factors (6), etc. We term the consequentially emerged or vanished binding motifs of important regulator molecules as ‘somatic motifs’.

TF motifs are an indispensable element for fueling the transcription machinery; thus, TF somatic motifs attract the most intensive research attention. One extensively analyzed TF binding mutation in human cancers is located in the TERT promoter region, which creates a new canonical binding motif TTCCGG for oncogenic E26 transformation-specific (ETS) factors; this gained motif allows ETS proteins to bind to the mutated promoter to trigger TERT expression, resulting in uncontrolled cell proliferation and eventual tumorigenesis (7).

RBPs bind to the double- or single-stranded RNA in cells through recognizing specific RNA recognition motifs. RBPs have been found to play important roles in the post-transcriptional gene regulation process, and their impact on cancer biology has been well documented (8). Henceforth, the binding motifs of RBPs, primarily located in 3′-UTRs and introns, become another major source of somatic motifs.

miRNAs are a type of small non-coding RNAs, known for their ability to regulate protein-coding genes via complementary binding to the seed regions (six to eight nucleotides from the 5′ end of miRNAs). Somatic mutations in the seed sequences can cause significant deviation from normal miRNA–mRNA regulation networks. For example, it has been shown that mutations in the seed region of miR-96 are responsible for non-syndromic progressive hearing loss (9). Similarly, a mutation in the seed region of miR-84 causes EDICT syndrome (10). miRNA typically binds to 3′-UTR of mRNA (6). A somatic mutation in the 3′-UTR binding region can also disrupt normal miRNA–mRNA

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binding. Substantial efforts have been made to curate and document somatic mutations in miRNAs and their impact, such as the SomamiR database (5).

We extend the source of somatic motifs from somatic mutations to two additional sequence alteration mechanisms: RNA editing and germline inherited single nucleotide polymorphisms (SNPs). RNA editing is an enzymatic modification process that alters RNA molecule’s nucleotide sequence in relation to the corresponding DNA sequence. RNA editing events mainly come in the form of adenine-to-inosine (A-to-I) and cytosine-to-uracil (C-to-U) substitutions, with the former taking an overwhelming (>90%) dominance (11). Recent studies have revealed significant such as the SomamiR database (5).

Substantial efforts have been made to curate and bind. SNPs can affect disease risk and regulate gene expression as evidenced by the thousands of genome-wide association studies and gene expression quantitative trait loci (eQTL) studies. It has been suggested previously that eQTLs regulate gene expression by affecting TF motifs (15), which indicates that SNPs can affect the binding efficiency if one of the two alleles creates a new binding motif. We hypothesized that SNPs may also affect the binding efficiency of RBPs and miRNAs with similar mechanisms, which can be used to explain a large portion of the cis-eQTL regulation mechanism.

As we articulated above, somatic motifs can be ascribed to a variety of sequence alteration mechanisms and they may take place in motifs of all kinds of molecular regulators. Somatic motifs have been studied sporadically with a focus on high potential targets such as TERT promoter mutations (7,16). However, somatic motifs have not been curated into a comprehensive resource. Furthermore, previous studies were usually focused on single nucleotide mutations, but have not yet analyzed insertions and deletions sufficiently. In this work, by analyzing multi-omic data of over 30,000 subjects from several large consortiums, we identified millions of somatic motifs ascribed to a variety of sequence alteration mechanisms in relation to TFs, RBPs and miRNAs. These somatic motifs, including a large portion of novel ones, were compiled into somatic motif database (SMDB) for easy searching, browsing and downloading by the research community at large.

MATERIALS AND METHODS

Somatic motif detection algorithm

Binding sequence alteration due to either somatic mutations or RNA editing, or any nucleotide modification process can result in disease-promoting biological chain reactions. The overall algorithm of somatic motif detection algorithm is explained in Figure 1. Given a set of somatic mutations, and a list of target motifs (short nucleotide sequences), we detected whether somatic motifs are gained or lost as a consequence of the specified somatic mutations. The foremost step of our algorithm is to derive altered sequences that embed the provided somatic mutations. When somatic mutations occur in proximity to each other, the combinations of n nearby mutations lead to 2^n − 1 alternative somatic sequences that are existent in equal likelihood. Our somatic motif algorithm is capable of handling both small insertions and deletions. Both forward and reverse strands were considered for somatic motif identification.

Data acquisition

Two sets of somatic mutation data were downloaded. The first set contains 10,182 subjects of 33 cancer types from The Cancer Genome Atlas (TCGA). The second set contains 19,729 subjects of 57 cancer types from 81 projects within the International Cancer Genome Consortium (ICGC).

RESULTS AND DISCUSSION

Web design and interface

Through our intense analysis of omic data from several large consortiums, we identified millions of known and novel somatic motifs from three major nucleotide sequence altering mechanisms: somatic mutation (single and INDEL), RNA editing and SNP (Figure 2). These somatic motifs were organized, annotated and placed into SMDB for further usage. SMDB was designed using MySQL and the web interface is constructed using PHP and JavaScript. All results in SMDB are based on GRCh38 human genome reference. The database can be categorized into three large categories: TF somatic motifs, RBP somatic motifs and miRNA-related somatic motifs. miRNA-related somatic motifs were further divided into miRNA seeds and miRNA–mRNA 3’UTR binding subsections. The queryable fields are genomic locations, motif type (gain or loss), motif sequence, project (available for ICGC and TCGA), mutation gene, motif gene, tissue type (available for GTEx), SNP ID, etc.
Somatic motifs associated with TF binding sites

TFs usually bind to gene regulatory regions such as promoters. While the promoter is generally considered as a non-coding region, TF binding sites play critical roles in regulating gene transcription. The TCGA somatic mutation data were derived from exome sequencing data, thereby resulting in insufficient coverage in non-coding regions including promoters. Thus, TCGA somatic mutation data are not suitable for large-scale detection of TF binding somatic motifs. In contrast, the ICGC somatic mutation data consist of whole genome sequencing data generated from numerous projects. Therefore, we analyzed 78 700 582 ICGC somatic mutations to identify altered binding sequences for the 746 TF motifs extracted from JASPAR. The JASPAR TF motifs range from 6 to 15 nucleotides with an average length of 12 nucleotides. Our initial analysis revealed 35 408 somatic motifs (15 932 gains and 19 476 losses) distributed across the 746 TF motifs. However, some of the TF motifs listed in JASPAR are not located upstream of protein-coding genes and thus may not be functional in regulating gene expression. The top high-frequency somatic motifs are displayed in Table 1 as an example of SMDB. They include 9 mutations that likely create new motifs (i.e. gain of functions) and 12 mutations that potentially deactivate TF motifs (i.e. loss of functions). Nineteen of the 21 somatic motifs are attributed to INDELs, while the other 2 are caused by single point mutations. Insertions contribute to a large portion of these somatic motifs. For example, in the biliary tract cancer Singapore cohort (BTCA-SG), the high-frequency (18.31%) insertion of [CCCCTCCCCC]CTT at the upstream of **RCOR3** gene forms a new binding motif sequence C[CCCCCTCCCCC] for **ZNF148**, a TF related to multiple cancer risks by regulating **TERT**, an oncogene encoding the telomerase reverse transcriptase (16). Deletions can contribute to the elimination of a binding mo-
Figure 2. (A) The overall results from conducting somatic motif analysis from five data sources (TCGA, ICGC, GTEx, dbSNP, REDIportal) against multiple data sources. The bar represents the log_{10} value of identified somatic motifs. (B) Genome-wide visualization of the TF somatic motifs in circos plot. There are four layers in this circos plot. The outer layer represents the genome by chromosome; the second layer (gold) represents somatic mutations that cause gains of TF motif; the third layer (dark slate gray) denotes somatic mutations that caused loss of TF motifs; and the fourth and inner layer (khaki) denotes the location of the binding motif gene. In the middle, the arrows’ color matches the layer’s color and is connecting the proper sequence altering mechanism on the second and third layers to its binding motif gene on the fourth layer. (C) Genome-wide visualization of miRNA–mRNA 3’-UTR bindingsomatic motifs. There are three layers in this circos plot. The outer layer represents the genome by chromosome; the second layer (chartreuse) represents the miRNA–mRNA 3’-UTR binding location; and the third and inner layer (khaki) represents miRNA locations. In the middle, the arrows’ color matches the layer’s color and is connecting the proper sequence altering mechanism on the second layer to its binding miRNA on the third layer.
than single nucleotide eQTLs. One of the interesting RBP practiceably, many of the top eQTLs are INDEL eQTLs rather
frequency. mutation-based somatic motif analysis identified 560 370 altered miRNA–mRNA 3′-UTR binding sequences. Somatic motif analysis using ICGC mutation data revealed 5484 261 somatic motifs (2 617 624 gains and 2 866 637 losses) associated with RBPs. However, no somatic motif occurs with a frequency >5% in our analysis. ICGC mutation-based somatic motif analysis identified 384 755 937 RBP somatic motifs (195 947 274 gains and 188 808 663 losses). eQTL somatic motif analysis on TF binding motifs shows that of the 47 876 unique upstream eQTLs, 3311 (~7%) caused gain or loss of TF binding motifs in JASPAR, which intuitively explains the SNP expression regulation mechanism.

**Somatic motifs associated with RBPs**

Next, we examined somatic mutations, RNA editing and SNP's effects on RBP motifs. We focused on RBP motifs using data in four major RBP databases [ATiRACT (21), oRNAMent (22), RBPDDB (23) and RBPmap (24)]. TCGA mutation-based somatic motif analysis identified 5 484 261 somatic motifs (2 617 624 gains and 2 866 637 losses) associated with RBPs. However, no somatic motif occurs with a frequency >5% in our analysis. ICGC mutation-based somatic motif analysis identified 384 755 937 RBP somatic motifs (195 947 274 gains and 188 808 663 losses).

dbSNP somatic motif analysis against RBP binding motifs revealed 1 478 913 372 somatic motifs (698 044 904 gains and 780 868 468 losses). GTEx cis-eQTL somatic motif analysis against RBP binding motifs identified 28 336 423 somatic motifs (14 050 215 gains and 14 286 208 losses). Noticeably, many of the top eQTLs are INDEL eQTLs rather than single nucleotide eQTLs. One of the interesting RBP target genes, SELENOF, a cancer-related gene in the folate metabolism pathway, has two intronic eQTLs that cause gain and loss of NOVA1 binding motifs. Of the 2 066 133 unique eQTLs in 3′-UTRs and introns, 2 065 869 (99.99%) have RBP somatic motifs and 1 265 570 (61.25%) have the same eQTL target genes as the RBP target genes. The regulation mechanism of these eQTLs can be intuitively explained by the gain or loss of RBP motifs due to the SNPs.

Initial somatic motif analysis of REDIportal RNA editing on RBP binding motifs from the four RBP databases revealed 181 568 somatic RBP motifs (64 414 gains and 117 154 losses). One RNA editing event can cause simultaneously loss and gain of binding motifs in the same RBP. For example, RBP ZFP35 has two binding motifs differentiated by one nucleotide (ACCTG[C] versus ACCTG[T]), according to the ATiRACT database. The RNA editing event on the reverse strand (T-to-C) at 3′-UTR regions of BPNT1 changes the sequence ACCTG[T] to ACCTG[C], which causes both gain and loss of ZFP35 motifs. Detailed results for RBP somatic motif analysis are not presented in the manuscript; they can be directly queried in SMDB.

**miRNA seed and target somatic motifs**

miRNAs regulate mRNA through their seed sequences. Somatic mutations occurring in the seed regions can substantially alter the mRNA targets. With ICGC and TCGA somatic mutation data, our somatic motif analysis detected 135 and 418 altered miRNA seeds in ICGC and TCGA, respectively. However, the majority of these altered miRNA seeds are caused by singleton mutations and none of these showed a frequency >5%. TargetScan (30) was used to predict mRNA targets for the original and new seed sequences. On average, the difference between the original seed targets and somatic seed targets is 70% for ICGC and 72% for TCGA. These results show that somatic mutations in miRNA seeds can lead to a substantial mRNA target shift. The biological effects of such mRNA target alterations have been demonstrated by previous studies (5,9,10).

RNA editing in miRNA seed regions has been shown to have a substantial impact on target mRNA selection and silencing efficiency (31). By applying RNA editing events in 17 TCGA cancer types to the SortmitMotif tool, we identified two somatic miRNA seeds. The A-to-I RNA editing event on position 63819626 of chromosome 9 caused miR-4477b seed TT[A]AGGA to become TT[G]AGGA, which affects ~66% of mRNA targets according to TargetScan’s prediction. This editing event was observed in 11 out of 17 TCGA cancer types with RNA editing data. The editing frequency ranges from 4.23% to 91.36% by cancer type.

Target mRNA 3′-UTR binding sequences for the miRNA seeds were obtained from starBase 2.0 (20). Somatic motif analysis using TCGA mutation data revealed 453 927 altered miRNA–mRNA 3′-UTR binding sequences. Somatic motif analysis using ICGC mutation data revealed 560 370 altered miRNA–mRNA 3′-UTR binding sequences. All 20 top hits are caused by INDELs. Many of the top hits have already been studied with miRNAs in cancers. For example, the highest mutation frequency (37%) ininsertion of T>TTT at chromosome 9, position 122847654 in the leiomyosarcoma French cohort occurred in the 3′-UTR region of RC3H2, which was recently found to facilitate cell proliferation by targeting miRNA miR-101-3p in oral squamous cell carcinoma. The second top hit with a 27% mutation frequency in the same cohort occurred in 3′-UTR of SRSF7, which has been shown to be target of multiple miRNAs and causes splice variants in renal cancer cells (32).
| Project | Chr | Location | Gene (upstream distance) | Mutation | Strand | Types | Affected motif (TF) | Frequency |
|---------|-----|----------|--------------------------|----------|--------|-------|---------------------|-----------|
| LMS-FR  | 8   | 18390725 | NAT2 (dist = 557)        | A > ATTA | +      | Gain  | [ATTA]AA (Arid3a)   | 11.94%    |
| LMS-FR  | 8   | 18390725 | NAT2 (dist = 557)        | A > ATTA | +      | Gain  | GTG[ATTA]A (H0XC4)  | 11.94%    |
| BTCA-SG | 9   | 93095871 | CARD19 (dist = 346)      | AAGGAAACCCCACCGGCCCCCCTTTACTCG > G | -      | Loss  | GCCCGGGCCC (KL5)   | 18.31%    |
| BTCA-SG | 7   | 101166514| VGF (dist = 945)         | C > CC   | -      | Loss  | CCTGCTGGC (ZEB1)   | 12.68%    |
| BTCA-SG | 1   | 21129205 | RCOR3 (dist = 72)        | C > CCCTCTCCCCCCTT | +      | Gain  | C[CCCTCTCCCCC] (ZN1F48) | 18.31%   |
| BTCA-SG | 1   | 21129205 | RCOR3 (dist = 72)        | C > CCCTCTCCCCCCTT | +      | Loss  | [C]GCCCTCTCCC (MAZ) | 18.31%    |
| MELA_AU | 8   | 56074582 | RPS20 (dist = 10)        | C > T    | +      | Gain  | T[T]CCTCGG (ETS1)  | 14.75%    |
| MELA_AU | 5   | 1295135  | TERT (dist = 67)         | C > T    | -      | Gain  | T[T]CCTCGG (ETS1)  | 11.48%    |
| BTCA-SG | 8   | 86514404 | RMDN1 (dist = 31)        | CA > C   | +      | Gain  | CGCCCCC[T]CCCCC (MAZ) | 12.68%   |
| LMS-FR  | 5   | 116090856| ARL14EPL (dist = 610)    | GTCTGG > G | -      | Gain  | TGC[G]T [ARNT]    | 16.42%    |
| LMS-FR  | 19  | 43826512 | ZNF283 (dist = 809)      | T > GTT  | -      | Loss  | TGC[GT]G (ARNT)    | 14.93%    |
| LMS-FR  | 19  | 14476342 | PTGER1 (dist = 988)      | T > GTT  | -      | Loss  | TGC[GT]G (ARNT)    | 13.43%    |
| BTCA-SG | 2   | 88056086 | KRR1C (dist = 303)       | T > TA   | +      | Loss  | A[T]TAAA (Arid3a)  | 14.08%    |
| BTCA-SG | 12  | 18090325 | RERGL (dist = 132)       | T > TA   | +      | Loss  | T[T]TAAA [AAAT] (ZNF384) | 11.27%   |
| BTCA-SG | 5   | 42812249 | SELENOP (dist = 173)     | T > TA   | +      | Loss  | T[T]TAAA [AAAT] (ZNF384) | 11.27%   |
| BTCA-SG | 17  | 81398932 | BAHCC1 (dist = 442)      | T > TA   | +      | Loss  | A[T]TAAA (Arid3a)  | 11.27%    |
| BTCA-SG | 6   | 15059621 | PLEKHI1 (dist = 264)     | T > TA   | +      | Loss  | A[T]TAAA (Arid3a)  | 11.27%    |
| LMS-FR  | 20  | 63490644 | EEF1A2 (dist = 561)      | T > TGGCGGT | -      | Gain  | TTCCGG [ETS1]    | 11.94%    |
| LMS-FR  | 2   | 68953575 | GKN2 (dist = 682)        | TGAA > T | +      | Gain  | A[T]TAAA (Arid3a)  | 17.91%    |
| LMS-FR  | 11  | 498223   | OR5L1 (dist = 750)       | TGGCTGT > T | -      | Loss  | TGGCTG [ARNT]    | 10.45%    |
| LMS-FR  | 11  | 498223   | OR5L1 (dist = 750)       | TGGCTGTGT > T | -      | Loss  | TGGCTG [ARNT]    | 10.45%    |

aChromosome. 
bGenomic location in GRCh38. 
*Mutation gene is the gene the somatic mutation occurred upstream to. The upstream distance is displayed in the parentheses.

dStrand where the somatic motif is observed: +, positive strand; -, negative strand. 
*The actual somatic motif sequence; nucleotide in brackets indicates the mutated position and nucleotide.

fMutation frequency = number of subjects with mutation/total number of subjects.
CONCLUSION

The identification of altered binding motifs resulting from somatic mutations or RNA editing has imminent scientific benefits. A myriad of studies have been conducted based on independent cases of such somatic motifs. Nearly all of them focus on the gain of somatic motif. We presented the first thorough SMDB and it stands out by identifying both losses and gains of important somatic motifs. The cascading biological effect from gain of an important motif is relatively easier to observe than the effect of loss of a motif. Because a binding sequence may have many targets, losing one may not cause a strong detrimental effect. However, some transcriptional effects can still be detected. For example, somatic mutations in the SDHD promoter region disrupted ETS binding motif and significantly reduced SDHD gene expression (33). We also identified this loss of motif in SDHD, except that this mutation did not meet the >10% mutation frequency threshold. Analysis using real somatic mutation data, RNA editing data and SNP data from large consortiums revealed some well-known and millions of novel somatic motifs. Many of the novel somatic motifs are of high frequencies deserving follow-up studies to examine functional mechanisms in more detail. By conducting large-scale analysis, we show that while some of the well-known somatic motifs have been studied, plenty of high potency targets await validation. Those high-frequency targets are curated in our database SMDB for easy search, browsing and bulk downloading.

DATA AVAILABILITY

The SMDB and the related resources can be accessed and downloaded at http://www.innovebioinfo.com/Database/SMDB/Introduction.php.

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