SOFTWARE TOOL ARTICLE

CusVarDB: A tool for building customized sample-specific variant protein database from next-generation sequencing datasets [version 1; peer review: 2 approved with reservations]

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Abstract

Cancer genome sequencing studies have revealed a number of variants in coding regions of several genes. Some of these coding variants play an important role in activating specific pathways that drive proliferation. Coding variants present on cancer cell surfaces by the major histocompatibility complex serve as neo-antigens and result in immune activation. The success of immune therapy in patients is attributed to neo-antigen load on cancer cell surfaces. However, which coding variants are expressed at the protein level can't be predicted based on genomic data. Complementing genomic data with proteomic data can potentially reveal coding variants that are expressed at the protein level. However, identification of variant peptides using mass spectrometry data is still a challenging task due to the lack of an appropriate tool that integrates genomic and proteomic data analysis pipelines. To overcome this problem, and for the ease of the biologists, we have developed a graphical user interface (GUI)-based tool called CusVarDB. We integrated variant calling pipeline to generate sample-specific variant protein database from next-generation sequencing datasets. We validated the tool with triple negative breast cancer cell line datasets and identified 423, 408, 386 and 361 variant peptides from BT474, MDMAB157, MFM223 and HCC38 datasets, respectively.

Keywords

Next-generation sequencing, Variant protein database, NGS-pipeline

Open Peer Review

Approval Status

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Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the **Oncology** gateway.

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Introduction

Cancer genome sequencing projects have revealed thousands of genomic variations in cancers (Forbes *et al.*, 2010; Tomczak *et al.*, 2015; Tate *et al.*, 2019; Zhang *et al.*, 2011). It is reasonable to presume that genomic variations in cancers might result in several new proteoforms. Genomic variations in coding regions may encode mutant proteins in cancer cells. These mutant proteins are known to drive proliferation in various cancers. For example, amino acid substitution of arginine to leucine at position 858 (L858R) in the epidermal growth factor receptor (EGFR) gene has been observed in 17% of pulmonary adenocarcinoma patients (Morgensztern *et al.*, 2015). In addition, a mutation in gene BRAF V600E is known to result in increased possibility of metastatic melanoma (Chapman *et al.*, 2011). Some of these mutant proteins are proteolytically processed in cancer cells, resulting in major histocompatibility complex (MHC) presentation of mutant peptides. These mutant peptides serve as neo-antigens that recruit T cells and result in immune activation (Kreiter *et al.*, 2015). Therefore, it is important to identify mutant proteins expressed by cancer cells. However, there are no easy-to-use pipelines for biologists to identify such coding variants, which alter the protein sequences and may play an important role in tumorigenesis. Detection of cancer-specific proteoforms has been studied by several research teams using proteogenomics methods. This approach integrates proteomics data with genome sequences to identify novel protein-coding regions (Menschaert & Fenyő, 2017; Nesvizhskii, 2016; Ruggles & Fenyő, 2016), which can be easily adopted in the field of cancer biology for the detection of genomic aberrations and coding single nucleotide polymorphisms (SNPs). Cancer is a heterogeneous disease with a large number of genomic variations. However, detection of mutations and SNPs at the proteomic level will aid in further investigations on altered molecular networks and functional ramifications and may provide candidate molecules for novel therapeutic interventions (Subbannayya *et al.*, 2016) for various types of cancers.

Several qualitative and quantitative proteomics studies have been reported, which identify altered expression of proteins in cancers (Subbannayya *et al.*, 2015). Often, general workflows were used in such investigations, wherein a reference protein database was used to search the experimentally derived tandem mass spectrometry data for the identification and quantification of the proteins (Kelkar *et al.*, 2014). However, such a reference database is usually deprived of sample-specific amino acid variations brought about by genomic aberrations and coding SNPs reported for the various cancers. The publicly available databases such as dbSNP (Sherry *et al.*, 2001), COSMIC (Forbes *et al.*, 2015) and UniProt (Apweiler *et al.*, 2004) can be used to identify the variant peptides (Alfaro *et al.*, 2017) but millions of protein-variants from these databases might increase the probability of identifying false positives. Therefore, there is a need for an improved specific method to characterize germline and somatic variations at the proteomic level (Alfaro *et al.*, 2017) specific to the cancer type. Hence, we developed CusVarDB with an in-built pipeline for genomics suite in deriving variants and creating custom variant protein databases.

Methods

Implementation

CusVarDB is available at <https://sourceforge.net/projects/cusvardb/> and <http://bioinfo-tools.com/Downloads/CusVarDB/V1.0.0/>. Our tool supports graphical user interface (GUI) for the easy execution of the next-generation sequencing (NGS) pipelines. The GUI was developed using Microsoft Visual Studio Community edition 2017. Linux commands were executed through the Python program (terminal.py) developed by Linwei available on [GitHub](#). A portable version of Perl was used to execute the script for downloading the annotation database and executing the variant annotation. A python script was used to generate the custom variant database; the scripts were made portable using [PyInstaller](#). The dry-run concept is implemented in the tool to customize the commands according to user need and run in batches.

CusVarDB inherits different NGS pipelines for genomics, RNA-Seq and exome-seq datasets. The tool performs the following steps: i) alignment of genomic data to a reference genome; ii) variant calling; iii) variant annotation; and iv) generate variant protein database (Figure 1). Burrows-Wheeler Aligner (BWA) is executed for alignment of genome and exome (Li & Durbin, 2009) data, while HISAT2 is used for RNA-Seq data (Kim *et al.*, 2015). Subsequent steps involving sample labelling, variant calling and annotation are performed using Picard, GATK (McKenna *et al.*, 2010) and ANNOVAR (Wang *et al.*, 2010), respectively. CusVarDB substitutes the amino acid variations from the previous steps to generate a custom variant protein database.

Operation

CusVarDB runs on Windows 10 system. It requires Linux Bash Shell and ANNOVAR tool to be enabled and installed by the user. Minimum system requirements include Intel i5 or i7, having at least four cores with 8 GB of RAM and 1 TB hard drive. High performance processors such as Intel i9 or Xeon and large quantity of RAM can enable faster execution of tasks.

The tool requires FASTA, FASTQ and dbSNP database as input files. FASTA file will be loaded at configuration panel and indexed using bwa index or hisat2-build command. The quality control panel loads the FASTQ file and produces a quality check report using the [FastQC](#) tool. The third panel asks the user to load the FASTQ file(s), set the number of threads and RAM to perform the alignment and variant calling steps. This step produces the raw VCF file, which will be used for annotation in annotation tab. The annotation panel annotates the variants and incorporates those variants to the protein database to create a custom variant protein database. Detailed information of tool installation and execution of a test dataset are available in the manual (Manual.pdf) available to download [here](#) and on Zenodo (Kasaragod *et al.*, 2020b).

Use cases methods

The genomics datasets downloaded from the SRA repository were subjected to a quality check using the [FastQC](#) tool. Poor

Figure 1. Schematic representation of the workflow.

quality reads with Phred score less than twenty were trimmed using `fastx_trimmer`. Trimmed reads were mapped to [human reference genome version 19 \(hg19\)](#). `AddOrReplaceReadGroup` and `Markduplicates` operations from `GATK` were performed to add label information and to remove polymerase chain reaction (PCR) artefacts. Further, post alignment processing steps such as `IndelRealigner` and `BaseRecalibrator` were performed to correct the mapping errors made by alignment tool to increase variant calling accuracy. Variant calling was performed using `HaplotypeCaller`. Finally, raw variants were annotated using `ANNOVAR` and the annotated variants were incorporated to the protein database to create custom variant protein database.

Proteomic searches were carried out using Proteome Discoverer 2.3 (Thermo Scientific, Bremen, Germany). Searches could

also be carried out using freely available open source alternatives such as [MaxQuant](#), [MsFragger](#), [MS-GF+](#), [MyriMatch](#) or [OMSSA](#). Mass spectrometry raw files were obtained from the PRIDE archive ([PXD008222](#)) and searched against the customized variant protein database using the SEQUEST-HT search algorithm. Trypsin and LysC were set as proteolytic enzymes with a maximum missed cleavage of one. Carbamidomethylation of cysteine was set as a fixed modification, and acetylation of protein N-terminus and oxidation of methionine were set as variable modifications with a minimum peptide length of seven amino acids. The precursor mass tolerance was fixed as 10 ppm, and 0.05 Da for fragment ions. The mass spectrometry data were searched against the decoy database with a 1% false discovery rate cut-off at the peptide level.

We have provided a dataset for users to test the tool. The test dataset was taken from the study [SRR7418758](#) archived on the NCBI Sequence Read Repository (SRA) and aligned using [human reference genome version 19 \(hg19\)](#). Using `samtools` view command, reads mapped to chromosome 22 were extracted and converted to FASTQ files (paired-end). We have also provided chromosome 22 nucleotide sequences from hg19 and corresponding variant information from [dbSNP database](#).

Use cases

To determine the functionality of our tool, we have taken exome ([Daemen et al., 2013](#)) and proteome datasets ([Lawrence et al., 2015](#)). We incorporated 12,429; 13,923; 12,386 and 11,600 non-synonymous SNPs from BT474 (accession number [SRR925752](#)), MDMAB157 (accession number [SRR925788](#)), MFM223 (accession number [SRR925796](#)) and HCC38 (accession number [SRR925778](#)) cells, respectively, to the protein database ([Figure 2A](#)). These non-synonymous SNPs were incorporated to the reference protein database (Human RefSeq release 93) to create the customized variant protein database. The mass spectrometry-based raw files were searched against their respective custom variant protein database, which resulted in the identification of 423, 408, 386 and 361 variant peptides from BT474, MDMAB157, MFM223 and HCC38, cell line datasets ([Figure 2B](#)). The resultant variant peptide lists are available as *Underlying data* ([Kasaragod et al., 2020a](#)). The data generated from the tool demonstrates the usefulness and the ease of detection of variant peptides in an integrated omics analysis.

Conclusions

CusVarDB generates customized variant protein database from NGS datasets. To our knowledge, this is the first tool which uses the Linux Bash Shell to execute the NGS tools on a Windows OS. The tool provides the additional feature of dry-run, making the commands highly customizable. The variant protein database generated by this tool is highly compatible for use as a reference protein database for analysis of variants at the proteomic level.

CusVarDB

Figure 2. A) Number of nonsynonymous variants incorporated to protein database. **B)** Mutant peptides identified across four cell lines (BT474, MDMAB157, MFM223 and HCC38) where the mass spectrometry raw files were searched against custom mutant protein database.

Data availability

Source data

Whole exome data from [Daemen *et al.* \(2013\)](#) on BioProject, Accession number [PRJNA210427](#)

Mass spectrometry proteome data from [Lawrence *et al.* \(2015\)](#) on PRIDE, Accession number PXD008222: <https://identifiers.org/pride.project:PXD008222>

Test dataset on SRA, Accession number SRR7418758: <https://identifiers.org/insdc.sra:SRR7418758>

Underlying data

Zenodo: CusVarDB: A tool for building customized sample-specific variant protein database from Next-generation sequencing datasets. <http://doi.org/10.5281/zenodo.3747108> ([Kasragod *et al.*, 2020a](#))

This project contains the following underlying data:

- Gowda_CusVarDB_Supplementary_table1.xlsx (This table contains the resultant variant peptides along with

the wild-type peptides from BT474, MDMAB157, MFM223, and HCC38 datasets. Along with mutant peptides, this section also provides additional information such as peptide-spectrum match [PSM], Protein accession, cross-correlation value from the search [Xcorr] and retention time [RT])

- Gowda_CusVarDB_Supplementary_table2.xlsx (This table provides the complete details of the resultant peptides. Here the mutant and corresponding wild-type peptides are mentioned in different sheets. For a given mutant peptide its wild-type peptide and corresponding information can be mapped using the VLOOKUP function in Excel by keeping column A [SI.No] as lookup parameter.)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Software availability

Software available from: <https://sourceforge.net/projects/cusvardb/> and <http://bioinfo-tools.com/Downloads/CusVarDB/V1.0.0/>

Source code available from: <https://github.com/sandeepkasaragod/CusVarDB>

Archived source code at time of publication: <https://doi.org/10.5281/zenodo.3780645> (Kasaragod *et al.*, 2020b)

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Version 1

Reviewer Report 26 August 2020

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Richard Kumaran Kandasamy

Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

The manuscript by Kasaragod *et al.*, describes a software application for developing sample-specific protein sequence database that takes into account the protein-coding variants, for interpretation of mass spectrometry data to see what portion of the variants are seen at the protein expression level. This is an important aspect in exemplifying the use of genomic data on proteomics datasets associated with cancer samples.

The software is made available for download and enough documentation has been provided for potential users.

The authors use this data set to identify protein-level evidence for expression of variants in four different cancer cell lines.

The manuscript can be accepted for indexing. I have one important point that I would like the authors to address:

While the findings are potentially interesting, it would be nice if the authors pick a few important examples specific to these four cell lines.

This would give the users a better appreciation of the biological insights given by this application.

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow

replication of the software development and its use by others?

Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Partly

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research expertise include proteomics, proteogenomics, bioinformatics and systems immunology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 23 July 2020

<https://doi.org/10.5256/f1000research.25627.r63336>

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Suresh Mathivanan

Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Vic, Australia

This is a manuscript that describes an important tool.

1. The authors may need to discuss other proteogenomic tools similar to this and provide the advantages. If such tools don't exist, perhaps make a strong case. Zhang lab tools can also be discussed with references.
2. Can the authors also discuss and refer previous attempts on onco-proteogenomics? One of the first paper on onco-proteogenomics came in 2012: Mathivanan, S., Ji, H., Tauro, B.J., Chen, Y.S. and Simpson, R.J. (2012) Identifying mutated proteins secreted by colon cancer cell lines using mass spectrometry. *Journal of Proteomics*. 76, 141-9¹.
3. Does CurVardb provide only non-synonymous point mutations? What about frame shift mutations? Is that also provided or perhaps will be added in next version.

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Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer proteomics and bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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