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MI-PVT: A Tool for Visualizing the Chromosome-Centric Human Proteome

Bharat Panwar[†], Rajasree Menon[†], Ridvan Eksi[†], Gilbert S. Omenn^{*,†,‡,§}, and Yuanfang Guan^{*,†,‡,||}

[†]Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan 48109, United States

[‡]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109, United States

[§]Department of Human Genetics and School of Public Health, University of Michigan, Ann Arbor, Michigan 48109, United States

^{||}Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, Michigan 48109, United States

Abstract

We have developed the web-based Michigan Proteome Visualization Tool (MI-PVT) to visualize and compare protein expression and isoform-level function across human chromosomes and tissues (<http://guanlab.ccmb.med.umich.edu/mipvt>). As proof of principle, we have populated the tool with Human Proteome Map (HPM) data. We were able to observe many biologically interesting features. From the vantage point of our chromosome 17 team, for example, we found more than 300 proteins from chromosome 17 expressed in each of the 30 tissues and cell types studied, with the highest number of expressed proteins being 685 in testis. Comparisons of expression levels across tissues showed low numbers of proteins expressed in esophagus, but esophagus had 12 cytoskeletal proteins coded on chromosome 17 with very high expression (>1000 spectral counts). This customized MI-PVT should be helpful for biologists to browse and study specific proteins and protein data sets across tissues and chromosomes. Users can upload any data of interest in MI-PVT for visualization. Our aim is to integrate extensive mass-spectrometric proteomic data into the tool to facilitate finding chromosome-centric protein expression and correlation across tissues.

Graphical abstract

*Corresponding Authors: (G.S.O.) gomenn@umich.edu. Phone: +1-734-763-7583. Fax: +1-734-615-6553; (Y.G.) gyuanfan@umich.edu. Phone: +1-734-764-0018. Fax: +1-734-615-6553.

Notes

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Keywords

Michigan Proteome Visualization Tool; MI-PVT; chromosome 17; expression; testis; esophagus

INTRODUCTION

The aim of the Chromosome-Centric Human Proteome Project (C-HPP) of the Human Proteome Organization (HUPO) is to identify, map, and annotate all protein products of each protein-coding gene by chromosome through global efforts.^{1–3} The haploid set of human chromosomes contains 2.9 billion DNA base pairs^{4,5} and 20 055 protein-coding genes, according to neXtProt version 2014-09-19.⁶ With recent advances in high-throughput mass-spectrometry (MS) and RNaseq technologies, evidence is accumulating that the total number of protein species is much higher than that for genes because of alternative splicing,⁷ post-translational modifications,⁸ proteolytic modifications,⁹ and genetic variations.^{10,11} It is important to know the expression profile of proteins in order to understand their functional aspects, but major complications in this arise from the different experimental protocols and technological platforms employed.¹² It is a challenge to capture and visualize information about the expression levels of all proteins, by chromosome of their coding genes, in many tissues. Many databases have been developed for managing proteomics data, such as neXtProt,⁶ PRIDE,^{13,14} PeptideAtlas,¹⁵ GPMDB,¹⁶ Human Protein Atlas,^{17,18} Proteinpedia,¹⁹ and ProteomicsDB.²⁰ Quantitative expression data can be generated from MS spectra. As proof of principle, we utilized the Kim et al. Human Proteome Map (HPM) MS data set (PXD000561) comprising 17 adult tissues, 7 fetal tissues, and 6 hematopoietic cell types, all analyzed by the same methods, instruments, and bioinformatics team.²¹

Many chromosome-centric tools have been developed by C-HPP teams for understanding and visualizing human proteomic data. The Proteome Browser Web Portal is an open-source resource from the chromosome 7 team in Australia for data integration and analysis.²² The Chinese C-HPP chromosomes 1, 8, and 20 Consortium has produced CAPER1.0, 2.0, and

3.0, a chromosome-assembled human proteome browser with a configurable workflow and cloud-based system for analysis of C-HPP data sets, including detection of novel peptides, exon-skipping events, sample-specific single amino acid variants (SAAVs), and known missense mutations derived.^{23–25} GenomewidePDB is a gene-centric proteomic database from Korea (chromosomes 9, 11, and 13 teams), which integrates chromosome-based proteomic information as well as transcriptomic data and some other public databases.²⁶ H-Invitational Extended Protein Database (H-EPD) has been developed in Japan (chromosome 4 team) as a strategy to combine database-driven proteome research with transcriptomic data.²⁷ ProtAnnotator in Australia provides chromosome-based functional annotation information for missing proteins.²⁸ Annually, the Human Proteome Project provides metrics on its progress using neXtProt, PeptideAtlas, GPMDB, and Human Protein Atlas.^{29,30}

Our web server, MI-PVT, has been developed for flexible use in C-HPP and by the broader community, linked to neXtProt. Using the data from Kim et al.,²¹ we illustrate applications to expression levels, and we also implemented our tool for isoform-level functional annotation^{31–33} with the C-HPP chromosome 17 team, which has published a series of papers focused on the ERBB2 (Her2/neu) amplicon and splice variants in breast cancers.^{34–36}

MATERIAL AND METHODS

Data Set

We retrieved the information for the reported protein expression matrix matched to 17 294 genes in the draft Human Proteome Map (<http://www.humanproteomemap.org/>).²¹ We recognize that many proteins reported by Kim et al.²¹ and Wilhelm et al.²⁰ may be false positives, according to reanalyses by GPMDB and PeptideAtlas as well as a recent study suggesting a novel target decoy-based protein FDR estimation approach for large-scale data.³⁷ Use of the reported data permits a proof-of-principle application to be performed on quantitative protein expression data from many tissue types to demonstrate the visualization tool. The neXtProt database, release 2014-09-19, has been used for chromosome-based mapping of the HPM. In total, 16 635 proteins have been mapped successfully, with no information for the remaining 659 genes.

The neXtProt 2014-09-19 release annotated gene-centric proteins with different protein existence (PE) levels: 1, evidence at protein level (16 491); 2, evidence at transcript level without protein evidence (2647); 3, inferred from homology (214); 4, predicted (87); and 5, dubious or uncertain (616). After mapping 16 635 HPM proteins with neXtProt, we found that 15 075, 1420, 68, 22, and 50 proteins belong to protein existence levels PE1, PE2, PE3, PE4, and PE5, respectively.

Human Chromosomes and Tissues

Proteomes for autosomal chromosomes 1–22 as well as for the X, Y, and mitochondrial (MT) chromosomes have been analyzed from HPM. The HPM provided expression profiles of 17 adult tissues (adrenal, colon, esophagus, frontal cortex, gallbladder, heart, kidney, liver, lung, ovary, pancreas, prostate, rectum, retina, spinal cord, testis, urinary bladder), 7

fetal tissues (brain, gut, heart, liver, ovary, placenta, testis), and 6 primary hematopoietic cells (B-cells, CD4-cells, CD8-cells, monocytes, NK-cells, platelets). All of these tissues and cells were obtained from normal, healthy humans.

Protein Expression Profiles

Expression values have been directly used as final averaged spectral counts per gene per sample.²¹ These expression values vary from 1 to 16 589. To search proteins with different expression levels across tissues and chromosomes, MI-PVT offers an option to select different HPM-based expression levels based on spectral counts; we used <1, no expression; 1–3, low expression; 3–12, medium expression; and >12, high expression. We created three bins (33% of the values in each bin) for low, medium, and high expression. The bins can be adjusted to make tertiles or quartiles of the user's choice. Proteins with >1000 spectral counts are considered to be very highly expressed. These expression values are transformed into log₂ values in the graphs for better visualization. In the box plots of a graph, we used the average expression of proteins corresponding to a specific chromosome and tissue. We used MultiExperiment Viewer (MeV) to generate different expression-value-based heatmaps.³⁸

Expression Values and Functional Annotation

Detailed information on expression values and functional annotation is available via an X-axis hyperlink for both chromosome and tissue searches. Functional annotation of different proteins is essential for understanding the biological significance of protein isoforms. Earlier, we published many studies on the functional annotation of isoforms.^{31–33,39,40} Now, we adopted a similar methodology to predict the function of different human isoforms and implemented IsoFunc with the MI-PVT tool. The publicly available transcriptomic profiles used in Multiple-Instance Learning (MIL) and GO-ontology based functional annotations were assigned to isoforms.³⁹ Each predicted annotation has a fold-change value, which is calculated as the ratio of the rank probability of an isoform to the base probability.

RESULTS AND DISCUSSION

Chromosome-centric visualization of proteomics data is a challenge because of variation among tissues as well as the dynamic nature of protein expression in health and disease. Two major search options, (i) chromosome-based search and (ii) tissue-based search, are demonstrated in separate webpages. Here, we used chromosome 17 as an example to explain the utility of this tool.

Chromosome-Based Search

MI-PVT offers options for choosing the chromosome, choosing proteins of specific interest, selecting a different protein expression range, and choosing different PE levels. It provides results in a user-interactive mode, where tissue and protein information are displayed on the X- and Y-axes, respectively. Two different Y-axes have been used to show the number of proteins (spline chart) and average expression values (box plot). This graph gives an overview of the total number of proteins and their expression values across different tissues. As an example, more than 300 proteins from chromosome 17 are expressed in each of the 30

tissues and cell types studied (Figure 1). Three tissues (testis, retina, and ovary) and CD8 cells have more than 600 expressed proteins. The highest number expressed from chromosome 17 is 685 proteins in the adult testis, and the lowest number expressed is 330 proteins in adult esophagus.

Tissue-Based Search

Similarly, a tissue-wide search option has been implemented to visualize patterns of a particular tissue across different chromosomes. The *X*-axis displays tissue information, whereas protein abundance and expression values are shown in two different *Y*-axes. Figure 2 shows protein expression in adult esophagus for different chromosomes in a defined expression range.

Expression-Level Based Search

All expressed proteins vary in their expression level; therefore, we have provided an option to browse proteins according to their expression range across different tissues/chromosomes. For example, the lowest number of proteins expressed from chromosome 17 (330) is found in adult esophagus tissue, but when we browse only proteins with >1000 spectral counts, this tissue has 12 proteins in comparison to other tissues (Figures 3 and 4). Surprisingly, only one protein (ACTG1) of adult testis has a >1000 spectral count (Figure 3), whereas this tissue has the highest number of expressed proteins (685) from chromosome 17 (Figure 1).

Expression Profiles and Functional Annotation

Detailed information about the selected proteins can be retrieved by clicking on particular chromosome/tissue entries on the *X*-axis of the graph (Figures 1–3). It provides information about the gene, neXtProt accession ID, protein expression values (spectral counts), and IsoFunc-based functional annotations. IsoFunc is a human-specific version of our previously published method, IsoPred, for the functional annotation of mouse gene isoforms.³⁹

Next, we retrieved detailed information about the 12 proteins from chromosome 17 that are very highly expressed in adult esophagus (Figure 3). Interestingly, all 12 of those proteins are human cytoskeletal (actin, myosin, and keratin) proteins (Figure 4). In contrast, only one very highly expressed cytoskeletal protein (actin) was found in the testis. These cytoskeletal proteins play important roles in metastasis because migration/invasion of cancer cells in surrounding tissues is responsible for tumor spreading and is a major reason for poor prognosis.^{41,42}

Indeed, the uniformity of Kim et al. data across 30 different tissues and cell types provided an opportunity to perform chromosome-based comparative analysis. It is very difficult to perform such analyses with other very heterogeneous proteomic data sets in the public domain.

MI-PVT Web Server

We believe that the MI-PVT (<http://guanlab.ccmb.med.umich.edu/mipvt>) tool will be useful for protein scientists to explore the overall expression patterns of proteins coded by genes on specific chromosomes in various tissues. It will assist researchers in retrieving chromosome-

centric customized information in a user-friendly visualization format. There is an option available to choose any one *Y*-axis (either number of proteins or log₂ value of expression) at a time; downloadable and printable output result graphs are also generated. To increase the usability of MI-PVT, we have provided an option where the user can upload any data of interest. A sample file containing the expression of 906 transcription factors and protein kinases in 19 different tissues has been provided (Supplementary Table 4 from Wilhelm et al.²⁰). In the future, this tool will be integrated with several chromosome browsers from the Human Proteome Project and also will be expanded to compare MS and antibody profiling data. We will also provide positional information on chromosomes for the analysis of particular locations and amplicons and link the findings to isoform-level networks using our MIsoMine database.³³

CONCLUSIONS

MI-PVT has been developed as a tool for user-defined visualization of chromosome-centric and tissue-based human proteome data sets. It retrieves occurrence rate and quantitative expression information on proteins from user-defined chromosomes and tissues. We are hopeful that it will be useful among C-HPP teams and to the broader community as well as for scrutinizing public data.

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Figure 1. Expressed proteins from chromosome 17 in different tissues (highlighted in green for adult esophagus and red for adult testis; these tissues have the lowest and highest numbers of proteins expressed, respectively).

Figure 2.

Expressed proteins coded by genes on different chromosomes of adult esophagus tissues (highlighted in green for chromosome 17).

Figure 3. Expression and number of proteins with >1000 spectral counts from chromosome 17 in different tissues (adult esophagus is highlighted in red).

MI-PVT: Expression values (1000 to 16590) of proteins of Chromosome 17 in Adult_Esophagus tissue

Show 25 entries Search:

Gene	neXtProt AC	Protein Existence (PE) Level	Functional Annotation	Expression Value (Spectral Counts)
KRT13	NX_P13646	Evidence at protein level	IsoFunc	5319
MYH2	NX_Q9UKX2	Evidence at protein level	IsoFunc	3003
MYH1	NX_P12882	Evidence at protein level	IsoFunc	2371
KRT15	NX_P19012	Evidence at protein level	IsoFunc	2036
ACTG1	NX_P63261	Evidence at protein level	IsoFunc	1988
MYH8	NX_P13535	Evidence at protein level	IsoFunc	1938
MYH4	NX_Q9Y623	Evidence at protein level	IsoFunc	1721
MYH3	NX_P11055	Evidence at protein level	IsoFunc	1486
KRT14	NX_P02533	Evidence at protein level	IsoFunc	1369
KRT19	NX_P08727	Evidence at protein level	IsoFunc	1324
KRT16	NX_P08779	Evidence at protein level	IsoFunc	1223
MYH13	NX_Q9UKX3	Evidence at protein level	IsoFunc	1147

Showing 1 to 12 of 12 entries First Previous 1 Next Last

Figure 4.
Detailed output result of very highly expressed proteins of chromosome 17 in adult esophagus tissue, stimulating an exploration of their functional ramifications (see text).