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Integrated Bioinformatics Analysis of the Publicly Available Protein Data Shows Evidence for 96% of the Human Proteome

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Abstract

Protein-coding genes are predicted by genome annotation pipelines and are conceptually translated into protein sequences. Several thousands of these protein-coding genes catalogued in publicly-available databases seldom have evidence at the protein level. In this study, we have created a map of the human proteome by integrating publicly-available proteomic studies and resources. With the encompassed data, we are able to map 96% of the human proteome with ample experimental evidence for protein expression. Over 2.2 million annotations are recorded for 19,716 proteins from 63,239 independent studies that utilized more than 800 tissue/cell types/body fluids. Among the mapped human proteome, 96% of the protein expression is supported by two or more independent studies or experimental methods. The collated data (localization, tissue expression, post-translational modifications, protein-protein interactions, enzymes-substrate and 3D structures) is freely accessible through the web-based compendium Human Proteome Browser (http://www.humanproteomebrowser.info).

Keywords: Protein-coding genes; Human proteome; Genome annotation; Proteomic databases

Abbreviations: PTMs: Post-Translational Modifications; HGP: Human Genome Project; ESTs: Expressed Sequence Tags; HPP: Human Proteome Project; IHC: Immunohistochemistry; ORF: Open Reading Frame; TM: Transmembrane; HPB: Human Proteome Browser

Introduction

With the completion of the human genome project (HGP) [1,2], attention has now shifted to annotating the estimated 20,000 to 25,000 protein-coding genes [3,4]. The task of fully annotating the human genome is multifaceted involving first deciphering protein-coding regions of the euchromatin, followed by developing the definitive catalogue of encoded proteins (the proteome) and, finally, tying together literature information related to protein abundances, post- translational modifications (PTMs) and interacting partners in order to make the genome data useful to the broader biological research community. To this end, genome annotation pipelines have been developed and gene sequences (protein-coding and otherwise) are continuously catalogued in publicly-available sequence databases [5]. Eventually, all of these genes are conceptually-translated into proteins and are compiled in protein sequence databases. Though many genes have evidence of existence at the transcript level (mRNA, ESTs), relatively few have been identified at the protein level. On the other hand, some have neither transcript nor protein evidence and are merely predicted models. For example, Uni Prot [6], a protein sequence centric database, has protein proof only for 14% of its conceptually-translated protein sequences (at the time of writing). Another 13% have evidence at the transcript level only while a staggering 73% of the protein sequences have neither (http://au.expasy.org/sprot/relnotes/relstat.html). However, in the manually curated human Uni Prot KB/Swiss-Prot [6], the evidence of a gene at protein level is higher (~66%) but still not comprehensive.

Genome sequencing rates have drastically increased over the years and more than 3,800 organisms have already been sequenced [7]. In contrast, mapping and functionally annotating even one proteome is no trivial task [8,9]. We are yet to map the complete list of genes that are expressed at the protein level even for one organism [9,10]. Ten years on from the completion of HGP, there is still debate over the exact number of the estimated 20,000-25,000 protein- coding genes [3]. Unlike the finite genome, the proteome is very complex both temporally and spatially within a cell, depending upon physiological stimuli, epigenetic status, post- transcriptional and post-translational events [10,11]. Hence, functional characterization of each and every human protein based on their subcellular localization, tissue expression, isoforms, protein interaction partners, PTMs and 3D structures is indeed a daunting task.

To address this issue, an internationally-coordinated Human Proteome Project (HPP) was officially launched at the 9th Annual HUPO World Congress in September 2010 (http://hupo.org/research/hpp) to systematically characterize human proteins across various tissues and decipher their interaction partners by multiple proteomic methods [12]. Proteomic researchers have already initiated projects to catalog gene products at the protein level and to map the human proteome to its entirety [12-17]. One of the targets of the HPP is to develop a definitive catalogue of human proteins that can be experimentally identified at the protein level in various tissues/cell lines. In this endeavour, it is timely to catalogue extant protein identifications already present in the public domain – that is, to establish a reference starting point against which the HPP can be assessed. Decades of hypothesis-driven research has already been performed on human biological tissues and cell lines. While a bulk of these data are presently scattered across various publicly-available databases including BioGRID [18], Intact [19], Human Proteinpedia [20], HPRD [21], PRIDE [22], Peptide Atlas

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[23], Peptidome [24], HPA [25] Plasma Proteome Database [26], next Prot [27] and GPMdb [28], a substantial amount is hidden, and not queryable, in the supplementary tables of journal websites [20].

Here, we have created a list of 20,515 non-redundant human protein-coding genes (target proteome) by merging manually curated Uni Prot and Ref Seq [29] protein entries. Further, we collated extant human protein identifications to create the first draft of the human proteome. A three-pronged approach was used to accomplish the task: -I, integrating publicly-available proteomic databases, -II, collating published proteomic studies, and -III, undertaking protein- centric manual curation of scientific literature. Employing these strategies, we have assembled 2.2 million protein annotations from >800 tissues/cell types/body fluids and mapped 96% of the human proteome (compared against the target proteome). Among them, 96% were supported by two or more independent studies or experimental methods (e.g., mass spectrometry (MS), immunohistochemistry (IHC)) confirming the expression of the protein. Additionally, we were able to provide functional annotations for 19,716 proteins based on subcellular localization, tissue and cell line expression, PTMs, protein-protein interactions, enzymes-substrate relations and 3-D structures.

Methods

Creation of the human target proteome

To create a human proteome reference set, Ref Seq and Uni Prot human proteome sequences were combined together and the sequence accession numbers were mapped to Entrez Gene identifiers.

NP entries from Ref Seq human proteome database were mapped to Entrez Gene identifiers resulting in 19,129 unique genes. Similarly, Uni Prot human complete proteome was mapped to Entrez Gene identifiers resulting in 20,091 unique genes. However, 226 Uni Prot accession identifiers could not be mapped to Entrez Gene and were still added to the reference set. In total, combining Ref Seq and Uni Prot protein entries, the human target proteome set encompassed 20,515 unique genes.

Integration of proteomic databases

To integrate human protein data that is already available in proteomic databases, publicly accessible proteomic databases were used. Datasets were downloaded from various protein- protein interaction databases (BioGRID [18], Intact [19], Human Proteinpedia [20] and HPRD [21]) and mapped to Entrez Gene or Uni Prot accession identifiers. Customized Perl scripts were written to parse the respective datasets and only human proteins were retained in the final list. Similarly, protein structure (PDB [30]), mass spectrometry (PRIDE [22], Peptide Atlas [23], Peptidome [24] and Human Proteinpedia), immunohistochemistry (HPA [25] and Human Proteinpedia), protein annotation (HPRD, Entrez Gene and UniProt), exosome (Exo Carta [31], Vesiclepedia [32]), colorectal cancer (Colorectal Cancer Database), plasma (Plasma Proteome Database [33]), post-translational modification (Phosphosite Plus [34], HPRD, Human Proteinpedia and UniProt) databases were used to download the protein annotations and parsed with customized Perl scripts. As no two databases had the download files in same format or same accession identifiers, Perl scripts were customized to every single proteomic database file. The protein lists were collated and integrated into Human Proteome Browser.

Database cross reference file

Database cross references files were downloaded from NCBI Entrez Gene, Ref Seq, UniProt, IPI, Ensembl and GenBank. Protein accession identifiers were mapped to Entrez Gene identifiers and Uni Prot entries (for 226 entries that could be mapped to Entrez Gene ids). Unmapped protein identifiers were mapped again to Entrez Gene identifiers using DAVID and BioMart tools. The cross database mappings resulted in a master mapping file.

Data inclusion criteria

Various data inclusion criteria employed in Human Proteome Browser are as follows:

1. Experiment duplicates in various databases (based on the experimental accession number) were not considered.
2. Bioinformatics predictions were removed and protein identifications should be based on an experiment.
3. Orthologous protein identifications fixed to human proteins were not considered.
4. MS based subcellular localization datasets were used for protein identifications but were not used for subcellular localization unless another orthogonal method is used in the same study to prove the localization.
5. PTMs without amino acid sites were not considered.

Transcriptome

Microarray data for 79 human tissues was downloaded from Bio GPS [35]. Averaged mRNA expression values were used to perform hierarchical clustering for genes unidentified at the protein level. Customized R scripts were written to generate heat maps.

Proteotypic peptides

Peptide sequences from proteomic databases and published proteomic studies were searched using BLAST against human non-redundant database. Peptide sequences that uniquely identify a human protein were retrieved and were used in our analysis.

Results

Creation of a list of human protein-coding genes

It is not a trivial task to predict genes accurately from genomic data [36]. In *Mycoplasma genitalium* genome, 8% of the 340 genes were considered to be incorrect [37]. When the error rate is extrapolated for complex eukaryotic organisms with the consideration of intron-exon junctions, a significant number of gene predictions can be incorrect. Identification of an open reading frame (ORF) in genomic data does not always imply the existence of a protein-coding gene [36]. Apart from protein-coding genes, genome annotation pipelines predict a variety of gene types such as, pseudogenes, tRNA, rRNA, snRNA, scRNA, miscRNA and ncRNA. Irrespective of their protein-coding efficiency, all the gene types are conceptually-translated into corresponding protein sequences and stored in databases such as TrEMBL [38] (translation of EMBL nucleotide sequence database) resulting in several erroneous proteins [39,40]. Additionally, redundancy is also a concern [41] (TrEMBL currently hosts 76,802 human protein sequences). To provide reliable non-redundant protein-coding sequences, databases such as Ref Seq and Uni Prot adopted manual curation and, as a result, data is curated by highly trained biologists [40]. In this current study, to map the human proteome with extant protein annotations, a target set of non-redundant protein-coding genes that is curated by expert scientists is needed. Because Ref Seq and Uni Prot use different genome annotation criteria, these curated databases differ, to a significant extent, in their proteins [42]. For example, histone H3.1 is represented by 10 distinct genes (10 unique gene symbols) in Ref Seq (protein sequences are not always 100% identical) but only one entry (P68431) in UniProt. Similarly, human endogenous retoviral-K115 envelope protein (UniProt:Q902F8) is not found in Ref Seq. For this reason, we combined both Ref Seq and Uni Prot protein accession identifiers to create an integrated, non-redundant, list of human protein-coding genes – referred to as the 'target proteome'. To accomplish this, human complete proteome dataset from Uni Prot and NP entries from Ref Seq were mapped to NCBI Entrez Gene [43] identifiers resulting in 20,515 unique protein-coding genes (226 Uni Prot entries not mapped to Entrez Gene identifiers were retained in the target proteome). In the target proteome set (20,515 genes), 18,931 genes are common to both the curated versions while 198 and 1,386 genes are unique to Ref Seq and UniProt, respectively (Figure 1a).

Mapping the human proteome by three-phase strategy

To interrogate the extant literature for evidence of at least one protein product for every gene in the target proteome set, we used a three-phase approach -I, integrating publicly-available proteomic databases, -II, collating published proteomic studies, and -III, protein-centric manual curation of scientific literature (Figure 1b). In phase I, we integrated multiple proteomic databases as every database has a specific mandate and host data on particular features. PRIDE [22], Peptide Atlas [23] and Peptidome [24] are MS-centric while Bio GRID [18] and IntAct [19] are interaction specific databases. Currently, no compendium integrates heterogeneous data from various proteomic resources and presents it to the biomedical users. To integrate heterogeneous protein annotations, datasets were downloaded and parsed from a variety of proteomic resources including Exo Carta [44], PRIDE [22], Peptide Atlas [23], Human Proteinpedia [45], Peptidome [24], BioGRID, Human Protein Reference Database [46], Int Act [19], Uni Prot [6], Entrez Gene [43], PDB [30], Human Protein Atlas [25], Colorectal Cancer Database (Mathivanan, In preparation), Plasma Proteome Database [33] and Phosphosite Plus [34]. Redundant studies that are available in multiple proteomic databases were included only once, if they could be clearly separated. For example, Peptidome hosts MS experiments that are retrieved from Peptide Atlas and such studies were included once. As the download file formats, protein features annotated and the protein accession identifiers varied for each of the databases, individualized Perl scripts were used to parse the downloaded datasets. Protein accession identifiers were mapped to the target proteome by using a master database accession cross reference file that was created as part of this study. Additionally, tissue, localization and experimental method vocabularies were matched to community ontologies (eVOC [47], Gene Ontology [48] and PSI-MI [49]) and standardized terms were fixed for each entry. The collated protein annotations include subcellular localization, tissue and cell line expression, PTMs, protein-protein interactions, enzymes-substrate relations and 3-D structures. Notably, the dataset assembled here is obtained from multiple proteomic experimental methods and is not biased to any one particular method (e.g., MS or IHC). In phase I, by integrating proteomic databases, we identified 95% (19,552/20,515) of the target proteome while 963 (5%) proteins were not detected in any of the proteomic databases.

In phase II, we examined gene expression patterns of 963 genes (not identified in phase I) by downloading and examining mRNA

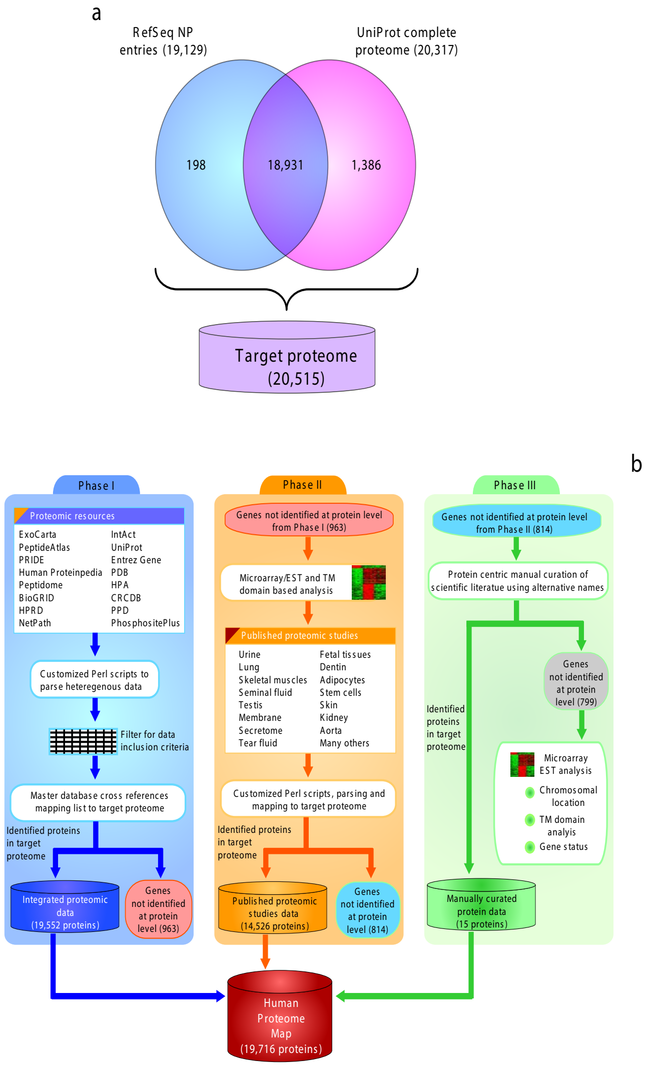

a
RefSeq NP entries (19,129)
UniProt complete proteome (20,317)
198
18,931
1,386
Target proteome (20,515)
b
Phase I
Proteomic resources
ExoCarta
PeptideAtlas
PRIDE
Human Proteinpedia
Peptidome
BioGRID
HPRD
NetPath
IntAct
UniProt
Entrez Gene
PDB
HPA
CRCDB
PPD
PhosphositePlus
Customized Perl scripts to parse heteregenous data
Filter for data inclusion criteria
Master database cross references mapping list to target proteome
Identified proteins in target proteome
Integrated proteomic data (19,552 proteins)
Genes not identified at protein level (963)
Phase II
Genes not identified at protein level from Phase I (963)
Microarray/EST and TM domain based analysis
Published proteomic studies
Urine
Lung
Skeletal muscles
Seminal fluid
Testis
Membrane
Secretome
Tear fluid
Fetal tissues
Dentin
Adipocytes
Stem cells
Skin
Kidney
Aorta
Many others
Customized Perl scripts, parsing and mapping to target proteome
Identified proteins in target proteome
Published proteomic studies data (14,526 proteins)
Genes not identified at protein level (814)
Phase III
Genes not identified at protein level from Phase II (814)
Protein centric manual curation of scientific literatue using alternative names
Identified proteins in target proteome
Genes not identified at protein level (799)
Microarray EST analysis
Chromosomal location
TM domain analyis
Gene status
Manually curated protein data (15 proteins)
Human Proteome Map (19,716 proteins)

Figure 1: Three phase approach to map the human proteome.

a) UniProt complete proteome and RefSeq NP entries were mapped to NCBI Entrez Gene identifiers and merged to create the target proteome set. 18,931 proteins were common between the two curated versions while 198 and 1,386 proteins were exclusively found in RefSeq and UniProt, respectively.

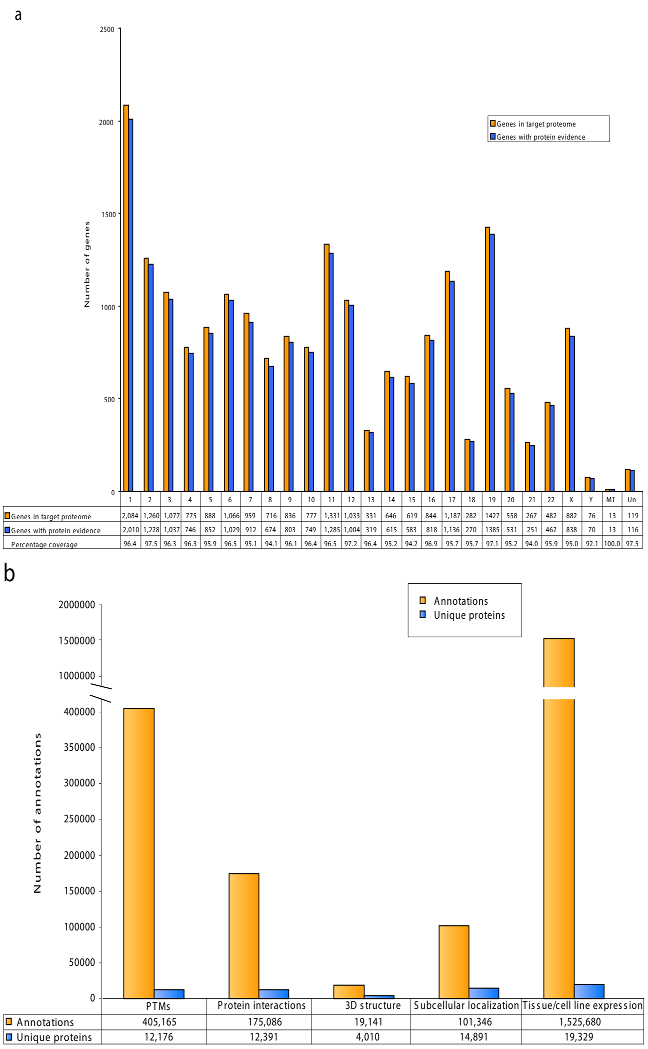
b) Mapping the human proteome was carried out in three-phases. In phase I, datasets were integrated from existing proteomic databases that host human protein data. Customized Perl scripts were used to parse the downloaded datasets and data inclusion criteria were followed. Filtered protein identifications were mapped to the target proteome and the resulting annotations were clustered for 19,552 unique proteins. In phase II, mRNA expression and TM domain were examined for 963 genes that were not identified at the protein level in Phase I. Tissues in which these genes are expressed were identified. Nearly 80 published proteomic studies (Supplementary Table 1) that use tissues/sample in which these genes are expressed were collated and integrated. All protein identifications from the proteomic studies that could be mapped to the target proteome resulted in 14,526 unique proteins, 149 of them were not detected in phase I. In phase III, manual curation of the scientific literature was performed for the remnant 814 genes that could not be identified at the protein level. An additional 15 proteins was collected in phase III. However, 799 genes could not be identified at the protein level by the three-phase approach. mRNA expression profiles and gene status were examined for these 799 genes to provide possible cluse on their protein-coding potentials. Protein data from the three phases were assembled to map the human proteome. The assembled data encompasses 19,712 unique proteins and is freely accessible through Human Proteome Browser (http://www.humanproteomebrowser.info). CRCDB – colorectal cancer database.

microarray-based expression profiles in 79 human tissues [35,50] and unigene-based EST expression datasets (http://www.ncbi.nlm.nih.gov/UniGene). The analysis revealed several tissues (fetal, skeletal muscles, testis, adipocytes, skin, kidney, developmental, lung and aorta) in which some of the 963 mRNA are expressed (data not shown). Proteomic experiments using some (e.g., testis, developmental tissues and skeletal muscles) of the identified tissues are minimal because of ethical reasons. Additionally, transmembrane (TM) domain based analysis confirmed the presence of several (24%) membrane proteins, a protein class that is often under-represented in proteomic studies due to low abundance and hydrophobhicity [51]. To capture the 963 genes not identified in phase I, proteomic experiments that used tissues in which some of the 963 genes were expressed and membrane enrichment studies were targeted. As a result, we collated nearly 80 proteomic studies (Supplementary Table 1) that were performed on testis, membrane molecules, skeletal muscles, cancer secretomes and bodily fluids. Supplementary and manuscript inline tables (few cases) were downloaded/copied and customized Perl scripts were used to parse these datasets. In a few instances, when data are not provided as supplementary information or when provided as a PDF file (which renders information retrieval harder), we approached the authors to obtain the protein identifications. Overall, the approach in phase II resulted in the identification of 14,526 proteins among which 149 were not detected in phase I.

A total of 814 proteins in the target proteome were not detected in phase I and II. In phase III, we performed manual curation of the scientific literature for the remaining 814 unidentified proteins by searching with available alternative names obtained from Entrez Gene and UniProt. Among these, 15 could be identified at the protein level by manual curation. Collectively, the three-phase strategy resulted in the identification of 19,716 proteins (i.e., 96% of the target proteome – Figure 1b). Chromosomal coverage of the mapped 19,716 genes with evidence at the protein level ranged from 92-97.5% (Figure 2a), the lowest in chromosome Y (70/76 proteins were detected – 92%).

Human Proteome Browser a compendium hosting heterogenous proteomic data

Proteomic data integrated as part of this analysis can be accessed freely through the compendium Human Proteome Browser (HPB) (http://www.humanproteomebrowser.info). Overall, 2.27 million annotations from 63,296 independent studies using 110 experimental methods are currently available in HPB (Table 1). As shown in Figure 2b, 405,165 annotations (12,176 unique proteins) are recorded for PTMs, 175,086 annotations (12,391 unique proteins) for protein interactions, 19,141 annotations (4,010 unique proteins) with 3D structures, 101,346 annotations (14,891 unique proteins) with subcellular localization and more than 1.5 million annotations (19,329 unique proteins) with tissue/cell line expression. Notably, 98% of the mapped human proteome had at least one tissue/cell line expression. Next, we examined which experimental method covered most of the human proteome. The most number of protein annotations (18,985 unique proteins) were recorded from MS (Figure 3a) where in 93% of the human proteome could be captured because of its high-through put nature [52]. Among the 18,985 proteins identified by MS, 18,646 (98%) proteins are detected by 2 or more peptides and/or supported by more than one study or experimental method emphasizing the high data quality. Notably, only 339 proteins were detected by single peptide and not supported by another study or experimental method. IHC had the second highest number of annotations largely derived from the Human Protein Atlas [25]. Among protein interaction methods, yeast-two hybrid based assay identified 8,710 proteins primarily due to proteome-wide interaction studies [53,54]. We further checked for the total number of proteins that are identified in cancerous tissues or cell lines. The cancer proteome assembled as part of this study includes 11,918 unique proteins (Figure 3b). Most number of proteins (10,643) is detected in ovarian cancer models followed by colorectal cancer (9,031). We next checked for the most often identified protein from decades of proteomics experiments. Albumin (ALB) surfaced as the most widely identified protein from 947 experiments followed by



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	MT	Un
Genes in target proteome	2,084	1,260	1,077	775	888	1,066	959	716	836	777	1,331	1,033	331	646	619	844	1,187	282	1427	558	267	482	882	76	13	119
Genes with protein evidence	2,010	1,228	1,037	746	852	1,029	912	674	803	749	1,285	1,004	319	615	583	818	1,136	270	1385	531	251	462	838	70	13	116
Percentage coverage	96.4	97.5	96.3	96.3	95.9	96.5	95.1	94.1	96.1	96.4	96.5	97.2	96.4	95.2	94.2	96.9	95.7	95.7	97.1	95.2	94.0	95.9	95.0	92.1	100.0	97.5

	PTMs	Protein interactions	3D structure	Subcellular localization	Tissue/cell line expression
Annotations	405,165	175,086	19,141	101,346	1,525,680
Unique proteins	12,176	12,391	4,010	14,891	19,329

Figure 2: Chromosomal and annotation feature based distribution of the human proteome.

a) Chromosomal distribution of 19,716 proteins is displayed. For every chromosome, total number of genes in target proteome, genes with protein evidence and the percentage coverage is shown as a table below the histogram. 119 proteins could not be mapped to any particular chromosome by the curated databases, among which, 116 proteins were identified at the protein level (97.5%). MT – mitochondrion; Un – unmapped to a chromosome.

b) Statistics of Human Proteome Browser data categorised by the annotation features is displayed. Total number of annotations and corresponding unique number of proteins for PTMs, protein interactions, 3D structure, subcellular localization and tissue/cell line expression is displayed as a histogram and table. Note that the units on y-axis (number of annotations) are altered to accommodate the tissue/cell line expression data.

	Data feature	Number
1	Protein annotations	2.27 million
2	Experiments	63,296
3	Unique proteins	19,716
4	Percentage of human proteome coverage	96%
5	Normal tissue/cell types/body fluids	704
6	Cell lines	85
7	Cancer tissues	18
8	Experimental methods	110
9	Proteins with subcellular localization	14,885
10	Proteins with tissue/cell line expression	19,329
11	Proteins with post-translational modifications	12,176
12	Proteins with 3D structures	4,010
13	Proteins with interactions	12,391
14	Proteins identified from all cancer tissues/cell lines	11,918

Table 1: Statistics of data integrated in Human Proteome Browser.

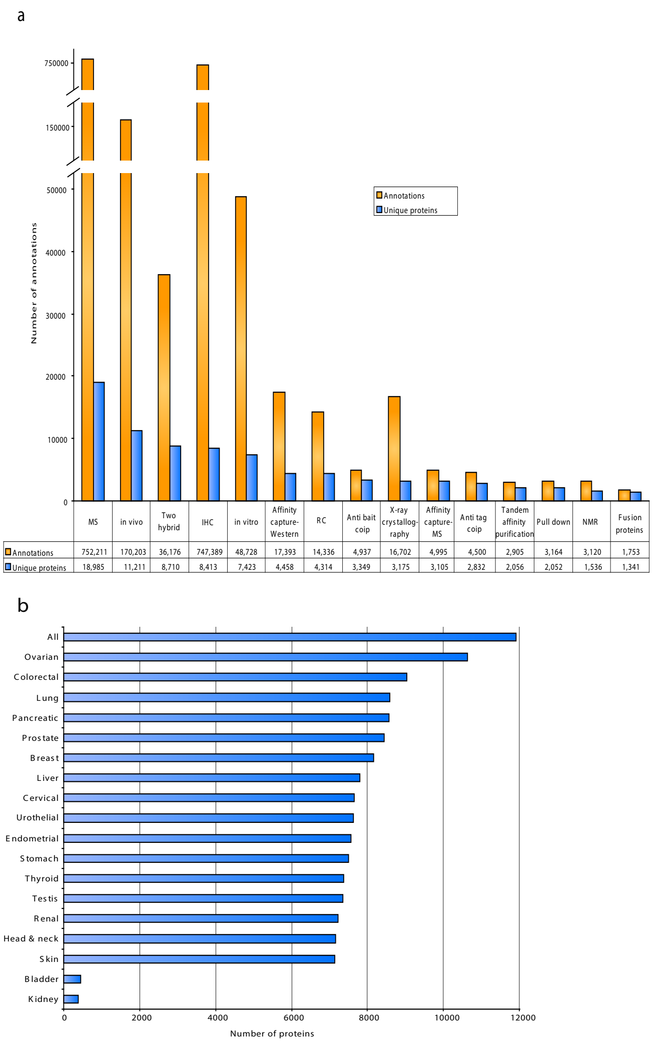
serpin peptidase inhibitor, clade A (SERPINA1) with 771 experiments. Overall, HPB integrates heterogeneous data under one single resource and biomedical users can access various features of their protein of interest in one website (Figure 4).

Data inclusion criteria and data quality

Even though some of the MS-based integrated datasets had a false discovery rate, it is hard to assign a false discovery rate for the integrated data as the collated data arises from multiple experiments including immunohistochemistry, Western blotting, confocal microscopy and MS. So the data inclusion criteria employed in our integration is critical to the data quality. To emphasise on high data quality, we had adopted various data inclusion criteria. First and foremost, only human protein data was included. Protein identifications mapped to human orthologs and any form of bioinfomatics predictions was omitted. For example, Gene ontology based subcellular localization profiles were downloaded from Entrez Gene and Uni Prot for proteins with experimental codes (e.g., IDA - inferred from direct assay; IPI – inferred from physical interaction; TAS – traceable author statement) and bioinformatics predictions (e.g., ISO – inferred from sequence orthology; IEA – inferred from electronic annotation) were discarded. Similarly, from PDB, 3D structures obtained by NMR and X-ray crystallography were retained while model-based predictions were removed. Similarly, MS based subcellular localization studies were used for tissue/cell line expression but were not considered for localization annotations due to the possible contaminations during fractionation procedures [55]. MS-based subcellular localization annotations were included when the data is validated by orthogonal experiments in the same study. By integrating multiple proteomic databases and studies, the confidence level of the protein identifications has eventually increased. One of the drawbacks of collating heterogeneous datasets is the increase in the individual false positives identifications. Nevertheless, in cases where multiple experiment platforms and independent studies support the protein identification, the integration of multiple resources can be justified. Based on this, 96% of the mapped human proteins were detected by two or more experiments/studies confirming the identification of the protein (Figure 5a). Eight five percentage of the proteins were detected by 5 or more experiments. A staggering 69% of the proteins are detected by 10 or more experiments again emphasising the reliability of the assembled data.

Majority of the proteins that were not identified were also not detected in the transcriptome dataset

In spite of our three-pronged approach to map the human proteome, 799 genes from the target proteome could not be detected at the protein level (Supplementary Table 2). No chromosomal location bias existed for the unidentified proteins (Supplementary Figure 1a). TM domain based analysis revealed that 22% of them have at least one TM domain (Supplementary Figure 1b). To check how many of the 799 genes have evidence at the transcript level, we examined the mRNA expression profile of these genes by using the downloaded 79 human tissue microarray dataset and UniProt-based transcript evidence. Interestingly, only 210 (26%) genes had expression at the transcript level while 74% (589/799) did not (Supplementary Figure 2). To check



	MS	in vivo	Two hybrid	IHC	in vitro	Affinity capture-Western	RC	Anti bait coip	X-ray crystallog-raphy	Affinity capture-MS	Anti tag coip	Tandem affinity purification	Pull down	NMR	Fusion proteins
Annotations	752,211	170,203	36,176	747,389	48,728	17,393	14,336	4,937	16,702	4,995	4,500	2,905	3,164	3,120	1,753
Unique proteins	18,985	11,211	8,710	8,413	7,423	4,458	4,314	3,349	3,175	3,105	2,832	2,056	2,052	1,536	1,341

Figure 3: Experimental methods and cancer proteome based distribution of the human proteome.

a) Statistics of the top 15 experimental methods that detected proteins is displayed. For every method, a table containing total number of annotations and unique proteins is shown below the histogram. MS had the most number of protein annotations (752,211) with 18,985 unique proteins (93% of the human proteome). Note that the units on y-axis (number of annotations) are altered to accommodate high numbers (MS and IHC). RC – reconstituted complex; coip – co- immunoprecipitation; Fusion proteins – fusion proteins for subcellular localization.

b) Statistics of cancer proteome is displayed. Unique number of proteins identified in various cancer tissues and cell lines is plotted as a bar graph. All represents the total cancer proteome (i.e., proteins identified in all cancerous tissues and cell lines). Ovarian cancer had 10,643 unique proteins expressed at various time points followed by colorectal cancer with 9,031 unique protein identifications.

for the protein-coding potentials, gene types of these curated genes were examined (Figure 5b). Among the 799 unidentified genes, 37% (297) are protein-coding with validated/provisional status, 24% (195) are protein-coding predicted models, 15% (116) are pseudogenes, 11% (89) are miscRNA, 11% (89) are uncertain/unknown models and 2% (13) are others. These results reveal that a majority of the unidentified genes potentially could be invalid protein-coding genes. It is possible that some of the valid protein-coding genes could be hidden in the scientific literature either in the main text or in the supplementary tables, which can't be queried. Perhaps some of them could be expressed only at certain stages in development or in specific tissues at certain time points. Further analysis need to be performed in order to add protein evidence to these molecules or retract them from the curated protein-coding list.

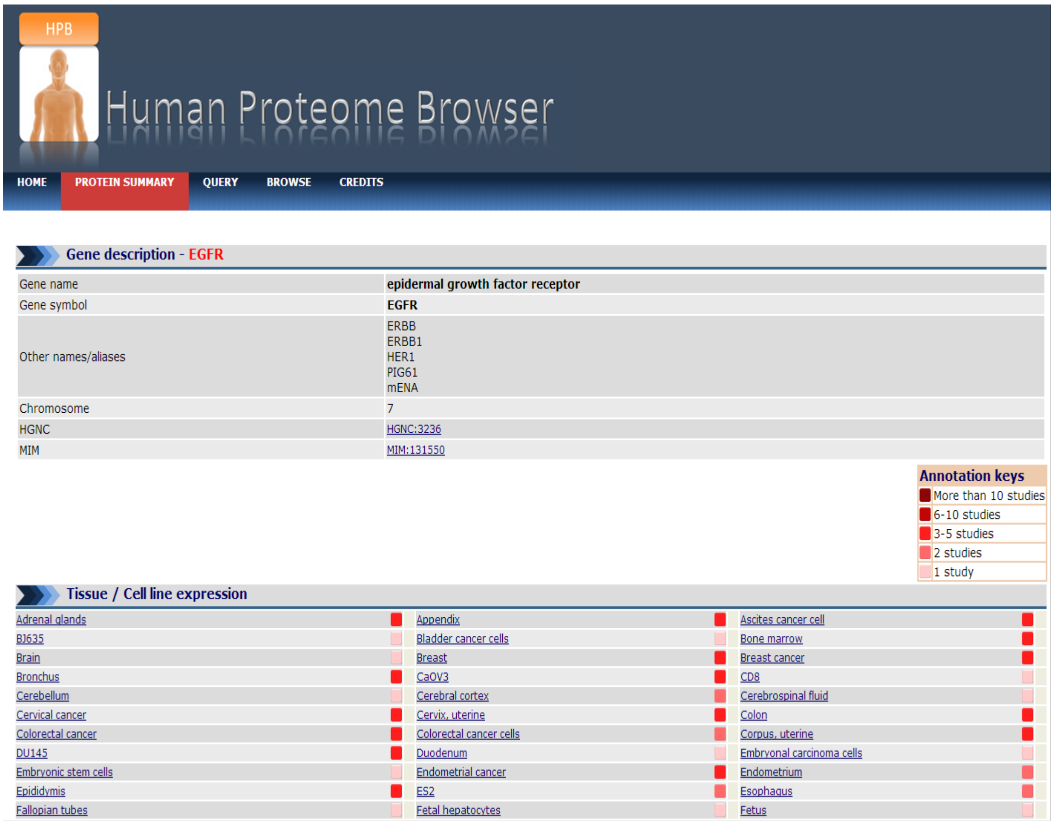
Beyond the target human proteome set

While a majority of the protein identifications from various datasets that were collated could be mapped to the target proteome, a minor set could not be mapped. Such unmapped protein entries can be categorized into two groups, -1, proteins that are deemed invalid and withdrawn from databases due to genome annotation pipelines, and -2, proteins that exist in non-curated databases but are not part of the curated versions. We analysed such unmapped proteins that do exist in non-curated databases but not in curated versions. 1,308 unique genes could be mapped from such entries (accessible through HPB). Among these, 1,208 were identified by MS and 671 (51%) are characterized as pseudogenes. Presumably, some of the peptides identified by MS could be identical to other valid protein-coding gene products. In order to check for bona fide protein identifications, we examined whether any of these proteins have been identified by proteotypic peptides (unique peptides that spans only one specific gene products). Interestingly, 297 proteins could be identified by proteotypic peptides. This clearly suggests that some of these protein identifications can be valid and the curation projects fail to catalogue them as bona fide protein-coding genes. For example, T cell antigen receptor alpha (TRA@) is detected by IHC, MS (5 independent studies), Western blotting, X-ray crystallography, in vivo and in vitro methods. Additionally, the membrane protein is detected by a proteotypic peptide (SDSYGYLLLQELQMK). Though some of these protein identifications can be valid, we emphasize caution in using these protein identifications as further experimental validations/manual curation need to be performed to confirm their protein-coding potentials.

Discussion

Pitfalls associated with large scale integration

Whilst the strength of the study is the integration of protein data obtained from multiple resources and heterogeneous experiment platforms, the weakness of the study is also the same where the assembled datasets can have false positives. Though the protein identification can be strengthened by multiple evidences, it cannot be ignored that individual false positive identifications still exist similar to other databases. In this study, we have mapped 96% of the human proteome and collated experimental evidence for the existence of 19,716



Gene description - EGFR

Gene name	**epidermal growth factor receptor**
Gene symbol	**EGFR**
Other names/aliases	ERBB ERBB1 HER1 PIG61 mENA
Chromosome	7
HGNC	HGNC:3236
MIM	MIM:131550

Annotation keys
■ More than 10 studies
▩ 6-10 studies
🟥 3-5 studies
▨ 2 studies
□ 1 study

Tissue / Cell line expression

Adrenal glands	🟥	Appendix	🟥	Ascites cancer cell	🟥
BJ635	□	Bladder cancer cells	□	Bone marrow	🟥
Brain	□	Breast	🟥	Breast cancer	🟥
Bronchus	🟥	CaOV3	🟥	CD8	□
Cerebellum	□	Cerebral cortex	▨	Cerebrospinal fluid	□
Cervical cancer	🟥	Cervix, uterine	🟥	Colon	🟥
Colorectal cancer	🟥	Colorectal cancer cells	▨	Corpus, uterine	🟥
DU145	🟥	Duodenum	□	Embryonal carcinoma cells	□
Embryonic stem cells	□	Endometrial cancer	🟥	Endometrium	▨
Epididymis	🟥	ES2	▨	Esophagus	▨
Fallopian tubes	□	Fetal hepatocytes	□	Fetus	□

Figure 4: A snapshot of Human Proteome Browser protein page.
EGFR protein page with tissue/cell line expression displayed from Human Proteome Browser. Biomedical users can browse heterogeneous protein annotations in one website, the Human Proteome Browser.

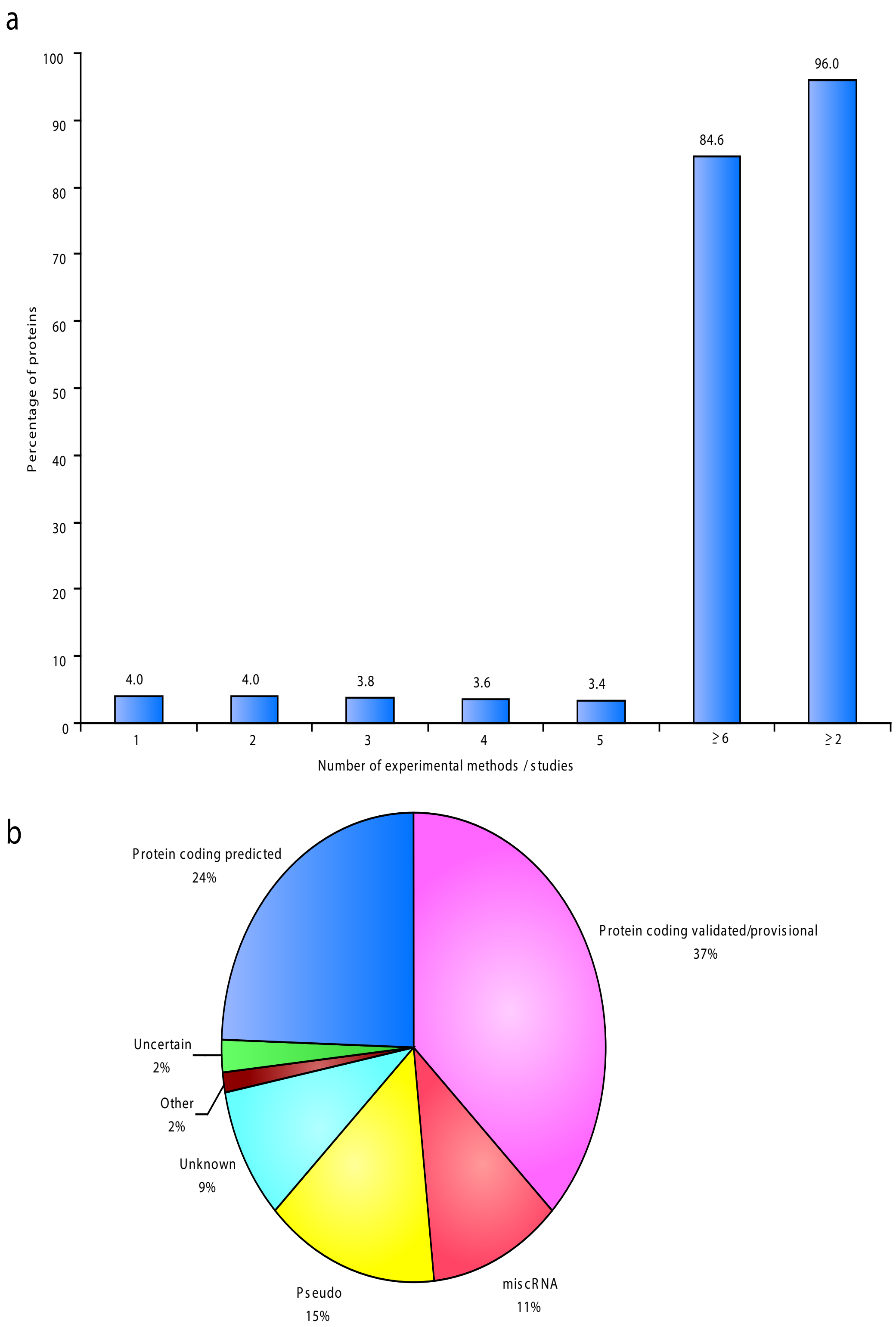


Figure 5: Distribution of proteins by number of experimental methods/studies and gene status.

a) Statistics of protein identifications in Human Proteome Browser by the total number of experimental methods or studies is displayed. Only, 4% of the proteins were identified by one experiment or study. A staggering 96% of the proteins were identified by 2 or more independent studies or methods while 84.6% of the proteins were identified by 6 or more.

b) Genes status distribution for 799 genes that could not be identified at the protein level is shown. 37% of the genes were denoted as protein-coding models with either provisional or validated RefSeq entries. 24% were predicted protein-coding models. The remaining 39% of the genes were denoted as pseudo, miscRNA, unknown, uncertain and others by the genome annotation pipelines.

genes at the protein level. We have indexed human proteins based on their subcellular localization, tissue/cell line expression, PTMs, protein interactions, 3D structures and enzyme-substrate relations. We believe that this study will be the one of the many that is aimed at deciphering the human proteome at various functional levels. HPP aims to characterize and quantitate every single protein in the human proteome. There are still a multitude of features yet to be unravelled in order to functionally characterize the entire human proteome. Protein isoforms, interaction partners for 8,000 proteins, protein abundances in various tissue and cell lines and finite number of protein-coding genes are yet to be documented.

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