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Perspective

Proteogenomic data and resources for pan-cancer analysis

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SUMMARY

The National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) investigates tumors from a proteogenomic perspective, creating rich multi-omics datasets connecting genomic aberrations to cancer phenotypes. To facilitate pan-cancer investigations, we have generated harmonized genomic, transcriptomic, proteomic, and clinical data for >1000 tumors in 10 cohorts to create a cohesive and powerful dataset for scientific discovery. We outline efforts by the CPTAC pan-cancer working group in data harmonization, data dissemination, and computational resources for aiding biological discoveries. We also discuss challenges for multi-omics data integration and analysis, specifically the unique challenges of working with both nucleotide sequencing and mass spectrometry proteomics data.

INTRODUCTION

Comprehensive molecular profiling is radically changing cancer research. Genomic catalogs of tens of thousands of tumors generated by The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) add immense depth to our understanding of mutations that drive tumorigenesis.[1] As sequencing on individual tumor cohorts are published, the next wave of manuscripts from these consortia examines patterns across cancer types to elucidate the context-dependent nature of mutations and their impacts.[2] One limitation of these sequencing-centric efforts is the paucity of data for proteins and their modifications. A few select proteins were monitored through antibody-based approaches such as reverse phase protein arrays (RPPA), but broad and unbiased proteomics data were not generated. As proteins represent the primary molecules responsible for metabolism, signaling, and structure, comprehensive and quantitative protein measurements are an essential part of phenotypic characterization. To connect genotype to phenotype, a true proteogenomic approach is needed.[3]

Proteogenomics analysis is a powerful method for discovering the next generation of precision treatments for cancer as it explicitly links genomic mutations to their impact on cellular physiology.[4–6] Early work by the Clinical Proteomic Tumor Analysis Consortium (CPTAC) demonstrated extensive proteome coverage with TCGA samples,[7] but also identified that sample collection protocols for TCGA allowed significant ischemia prior to tissue freezing. Thus the phosphorylation data measured in these tumors represented a mix of cancer-related and ischemia-related signaling.[8] As aberrant cellular signaling is an important hallmark of cancer dysfunction and ischemia activates several of the same pathways (e.g., MAPK signaling and apoptosis), it is necessary to create proteogenomic data from freshly acquired tumors with protocols designed to avoid ischemic artifacts.[9,10]

The CPTAC dataset currently includes 10 cancer cohorts of prospectively collected tumors analyzed with genomics, transcriptomics, proteomics, and phosphoproteomics (Figure 1). Molecular classifications derived from these primary data types are also available, e.g., HLA typing, immune cell decomposition, and ancestry prediction. Other protein post-translational modification (PTM) data such as acetylomics and glycoproteomics were generated for select cancer types. Standard clinical/demographic data and histology images have also been made available. Distributions of sex, age, tumor grade, tumor stage, smoking history, and recurrence status are illustrated in Figure 2. Detailed information of sample provenance is given in Tables S1 and S2. In the original publications investigating a single cancer cohort,[11–20] data were processed and analyzed by disease-specific working groups using customized genomics and proteomics data analysis pipelines. Therefore, to enable pan-cancer integrative analysis, and for consistency and reproducibility, we created a compendium of datasets where all proteogenomic data have been re-processed and harmonized.

Concurrent with this manuscript detailing the data processing and dissemination, CPTAC investigators have pursued biologically motivated pan-cancer analyses to illuminate mechanisms of cancer development. Pan-cancer investigation of protein post-translational modifications (PTMs) identified a subset of tumors with significant changes to cellular regulation, including dysregulated DNA repair, altered metabolic regulation associated with immune response, and patterns of acetylation that affect kinase specificity.[21] An integration of somatic driver mutations and proteomics data across tumor types resolves distinct cancer hallmark patterns.[22] Analysis groups continue to conduct thematic studies using the pan-cancer dataset described here, according to five identified themes: oncogenic drivers and pathways; DNA damage response; cell of origin; tumor microenvironment and immunotherapy; and clinical imaging, biomarkers, and actionable targets.

CPTAC datasets are generated as a resource for cancer research, and community-driven re-analysis is a positive and anticipated outcome from the program. Indeed, numerous groups have already begun re-examining the data.[23,24] They powerfully use proteogenomic data to reveal new molecular subtypes,[25–27] prognostic markers,[28–30] novel protein variants from alternative splicing and RNA editing,[31–33] and extensive post-translational regulation for protein complexes.[34,35] To facilitate an increased data reuse and serve the broad audience of cancer data stakeholders, we present our computational methodology for data harmonization and multiple dissemination mechanisms to share both the raw and processed data.

National Cancer Institute Data Commons

The Genomic Data Commons (GDC, https://portal.gdc.cancer.gov) and Proteomic Data Commons (PDC, https://pdc.cancer.gov) are National Cancer Institute (NCI) Cloud resources that coordinate storage and analysis of genomics and proteomics data for cancer research. The proteogenomic data generated by the CPTAC program is publicly disseminated through GDC and PDC, which host raw and processed data according to their in-house pipelines. As components of NCI Cloud resource, the GDC and PDC are fully integrated with other NCI Research Data Commons resources, e.g., the Cancer Imaging Archive (TCIA, https://www.cancerimagingarchive.net/), facilitating cloud-based analysis of proteomic, genomic, and imaging data. Driven primarily by the CPTAC projects, PDC organizes the data through a robust data model to maintain consistency and integrity of both data and associated metadata, and provides an interface to filter, query, search, and visualize proteogenomic data. A direct link to the harmonized data tables stored at the Proteome Data Commons is https://pdc.cancer.gov/pdc/cptac-pancancer.

Finally, in addition to thematic repositories, NCI's Cancer Research Data Commons contains a data type-agnostic resource, the Cancer Data Service (CDS). CPTAC has placed the processed and curated data files into the Cancer Data Service (CDS; https://dataservice.datacommons.cancer.gov/). The CPTAC data stored in the CDS includes all the harmonized proteogenomic data for our pan-cancer analyses, including mutation calls, RNA and protein quantification tables, clinical and demographic data, and derived molecular data such as HLA typing, immune cell decomposition, and ancestry prediction. The CPTAC pan-cancer data hosted in CDS is controlled data. Access to controlled access data on CDS is through the NCI data access policies approved, dbGaP compiled whitelists. Users can access the data for analysis with a queryable web

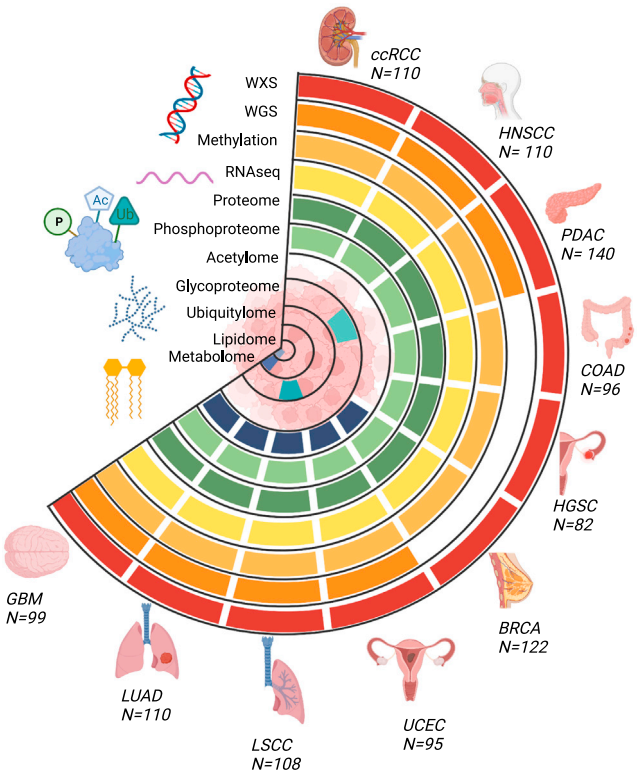


Figure 1. Tumor types and data types of the CPTAC pan-cancer dataset

Overview of the available molecular data types for the CPTAC pan-cancer cohort (n = 1072, see Table S1 for list of excluded cases and reasons for exclusion from the original datasets). Whole exome, whole genome, methylation, transcriptome, proteome, and phosphoproteome data are available for all ten cancer types. Normal samples are available for a subset of tumor types, see Tables S1 and S2.

portal through the Seven Bridges Cancer Genomics Cloud with dbGaP Study Accession, phs001287.v16.p6.

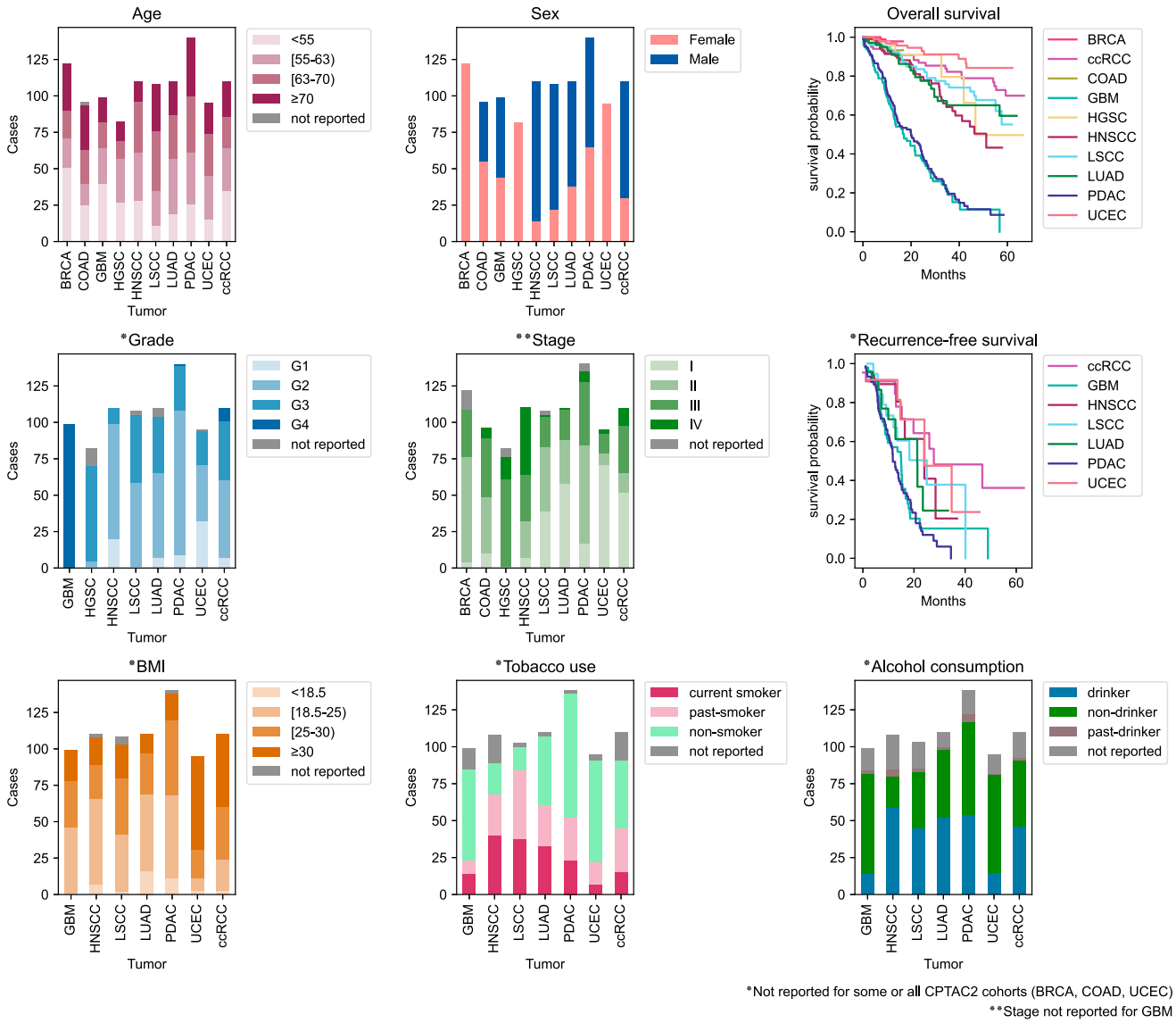
Data from multiple pipelines

Proteomic and genomic data analysis methods are continually evolving, and a variety of software tools exist for processing raw data into variant calls and quantifications (e.g., RNA or protein abundance matrices) that can be used for downstream analyses. As CPTAC consists of multiple groups with expertise in each data type, we have often analyzed data with multiple pipelines. Applying different tools to the same set of data may lead to different results and sometimes different conclusions. Therefore, benchmarking is important for tool assessment and selection. For somatic mutation calling, results from the ICGC-TCGA DREAM Somatic Mutation Calling Challenge show that different algorithms have characteristic error profiles, and an ensemble of pipelines always outperforms the best individual pipeline.[36] Based on this observation, and leveraging our team members' experience from the Multi-Center Mutation Calling in Multiple Cancers (MC3) project,[37] somatic mutation calling in our harmonized dataset was based on integrated results from the Broad Institute and Washington University in St. Louis pipelines, which each included multiple algorithms. RNA-seq data processing pipelines are now relatively mature with much overlap between widely used pipelines (e.g., https://nf-co.re/rnaseq). The major difference between the three pipelines used in this project is that the pipeline from Baylor College of Medicine includes circular RNAs in addition to linear RNAs. Quantifications for the vast majority of genes are not affected by circular RNAs and show very high correlation among the three pipelines. To compare different pipelines for proteomics data quantification, we have developed OmicsEV,[38] which uses more than a dozen evaluation metrics to comprehensively assess data depth, data normalization, batch effect, biological signal, platform reproducibility, and multi-omics concordance. Among the publicly available tools used by the CPTAC centers, the FragPipe pipeline usually provides higher data depth while maintaining similar or better performance for other metrics. Using three deep learning-derived features as evaluation metrics (predicted phosphosite probability, absolute retention time [RT] difference between observed and predicted RTs, and Pearson's correlation coefficient between observed and predicted spectra), we further found that FragPipe achieved higher sensitivity and quality for phosphopeptide identification and phosphosite localization compared with the other tested pipelines.[39] Based on these evaluation results, we provide one non-redundant, harmonized version with data across all cancer types and omics data types (see Baylor College of Medicine [BCM] pipeline for pan-cancer multi-omics data harmonization in Data S1 for details). However, we would like to emphasize that benchmarking is usually complicated by the lack of absolute ground truth, and thus more efforts should be put toward this important but challenging task. We have, therefore, also included results from multiple data processing pipelines in the data compendium. Users are encouraged to read the method description associated with each pipeline; explicit details can be found in the Data S1.

Programmatic Data Access

Simplifying data access can significantly remove barriers to community use and improve transparency and reproducibility. Therefore, CPTAC has created a software package that streams final quantitative data tables directly into a programming environment as dataframe variables (Figure 3). The Python application programming interface (API),[40] which originally streamed data from the individual cancer type publications, has been updated to provide access to the harmonized pan-cancer datasets described previously. Because data are streamed in native *pandas* dataframes, they are easily integrated with common machine learning and visualization packages such as SciKit-learn, PyTorch, Plotly, Seaborn, etc. Additionally, access to this API is also straightforward within R using the *reticulate* package for Python/R interconversion.

Computational APIs also extend the utility of CPTAC proteogenomic data by connecting them to other large public datasets.[41] We have recently expanded our popular R/Bioconductor tool, TCGAbiolinks,[42] to stream CPTAC pan-cancer data. In addition to leveraging the numerous software tools available within Bioconductor, TCGAbiolinks facilitates access to molecular data from TCGA, GENIE, MET500, GTEx, GEO, and IHEC. With TCGAbiolinks internal functions to harmonize data from diverse consortia, end-users can explore and validate hypotheses on a comprehensive library of reference datasets using sharable and reproducible codes.[43] See http://bioconductor.



*Not reported for some or all CPTAC2 cohorts (BRCA, COAD, UCEC)
**Stage not reported for GBM

Figure 2. Demographics of the CPTAC dataset

Distributions of selected clinical features among the pan-cancer cohort illustrated in Figure 1. Age is stratified by quartiles. Grade information is not available for BRCA and COAD cohorts. Stage information is not available for the GBM cohort. BMI, tobacco use, and alcohol use data are not available for BRCA, COAD, and HGSC cohorts. For survival plots, time starts at diagnosis. Additional clinical features, such as race and ethnicity, are available for exploration on the ProTrack pan-cancer sample dashboard.

org/packages/release/bioc/html/TCGAbiolinks.html for tutorials and instructions.

Web portals for data visualization and analysis

CPTAC teams have created several web portals for visualization and exploration of pan-cancer proteogenomics data (Figure 4). Each of these websites draws from the data compendium the appropriate datasets for pan-cancer analyses.

PepQuery

Cancer genomic studies have identified many genomic aberrations that may give rise to abnormal proteins, which are promising candidates for cancer biomarkers, drug targets, and neoantigens. Validation of their expression at the protein level is a critical step toward the clinical translation of these findings. PepQuery (http://www.pepquery.org) allows quick and easy proteomic validation of genomic aberrations, such as single nucleotide variants (SNVs), insertions and deletions (INDELs), RNA editing sites, novel junctions, fusions, and novel transcription regions, using MS/MS data.[44,45] We have recently introduced a new data indexing algorithm to improve the search speed and have expanded the dataset collection in the PepQuery web server to include MS/MS data from all 10 CPTAC studies, which increased the total number of MS/MS spectra to more than one billion.[46] Through the PepQuery web server and a mirror site at PDC (https://pdc.cancer.gov), users can directly query CPTAC and other MS/MS data with a novel peptide or DNA sequence of interest to look for supporting peptide spectrum matches (PSMs). For each PSM, annotated

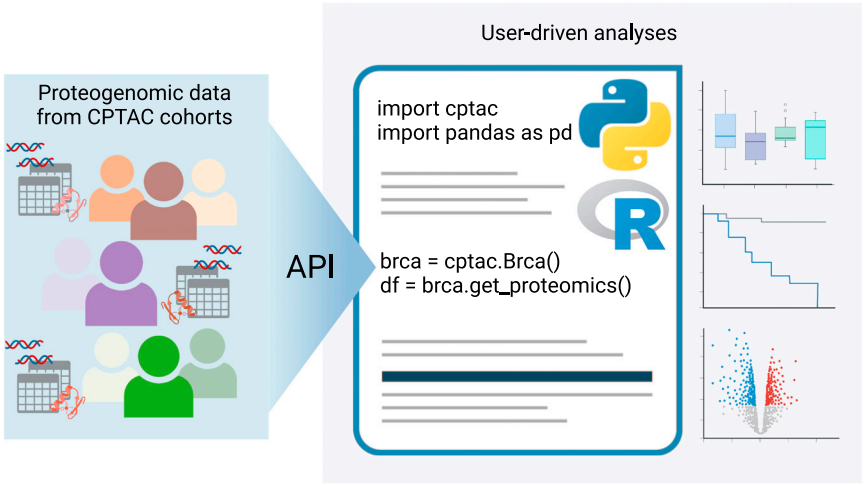


Figure 3. Streaming data with APIs
Programmatic access to CPTAC proteogenomic data across all cohorts is provided by both Python and R API.

spectra are provided for manual evaluation. Moreover, the stand-alone version and the implementation of PepQuery in the Galaxy Proteomics platform (https://proteomics.usegalaxy.eu/) support batch analysis and user-provided MS/MS data, and the identification results can be visualized using PDV.[47]

LinkedOmics and LinkedOmicsKB

LinkedOmics (http://www.linkedomics.org) is a data analysis portal that allows the characterization of any clinical or molecular feature of interest (e.g., survival, BRAF_V600E mutation, miR200c expression, or CHEK2-S422 phosphorylation) using cancer multi-omics data from TCGA and CPTAC.[48] We now provide the pan-cancer harmonized datasets described in this paper for all CPTAC cohorts in LinkedOmics. For each CPTAC study, the database stores data for >500,000 attributes including clinical attributes, mutations at site and gene levels, copy number alterations at region and gene levels, methylations at site and gene levels, mRNA expression, miRNA expression, protein expression, and PTM at site and protein levels. Using three analytical modules, including LinkFinder, LinkCompare, and LinkInterpretor, these data can be mined to reveal the consequences of genetic aberrations, characterize functions of genes and PTMs, and uncover molecular basis of cancer phenotypes.

The on-the-fly, user-defined data queries in LinkedOmics provide a high level of flexibility for analyzing CPTAC data, but performing data analysis on-the-fly is time consuming, and integrating and co-visualizing results from multiple cancer types and multiple omics data types remain challenging. To address these challenges, we further developed LinkedOmicsKB, a new knowledge portal that makes precomputed results for individual genes and phenotypes readily available through a single query.[49] All results for a query gene or phenotype are presented on a single page with user-friendly visualization to facilitate easy comprehension. The knowledge portal is available at https://kb.linkedomics.org.

PTMcosmos

PTMcosmos is an interactive web portal designed to catalog and visualize PTMs in humans. As a key regulator of protein activity, PTMs play an essential role in our understanding of cancer and dysregulated cellular states. The PTM sites detected across all CPTAC studies were harmonized using protein sequences from UniProt's reviewed proteome, allowing for the integration of extensive annotations from many established databases including the UniProt Knowledge Base, PhosphoSitePlus, and protein 3D structures. In total, we harmonized 210,112 PTM sites and annotated them with 11,265 publications. Additionally, to investigate the relationship between genetic alterations found in cancer and PTMs that are in close spatial proximity, we included cancer somatic mutations detected in the samples of CPTAC and TCGA. Finally, we developed interactive visualization tools to allow researchers to explore the existing literature on a PTM site, the difference in abundance between tumor and normal samples, and the PTM-mutation clusters on protein structures. PTMcosmos portal is publicly available at https://ptmcosmos.wustl.edu/.

ProTrackPath: pan-cancer portal

We have developed a web application for accessing pathway enrichment scores across the pan-cancer cohorts. While previous ProTrack applications allow users to visualize normalized raw data for individual cancers,[50–52] the ProTrackPath pan-cancer portal presents pathway enrichment scores across cancer types, calculated with a single sample gene set enrichment analysis (ssGSEA).[53] The user specifies a pathway database such as Hallmark,[54] KEGG,[55] or Reactome,[56] then selects a set of pathways to visualize. An interactive heatmap is then generated, which users can customize by sorting according to any given track or toggling categorical variables on and off. Additionally, the portal includes a sample dashboard view, which allows for viewing clinical characteristics. This allows users to explore the distributions of the cancer types along with various demographic and clinical features as bar graphs. Users can filter samples by toggling features in each bar graph's interactive legend, and then populate the heatmap with their custom-generated cohort. The portal is available to the public at http://pancan.cptac-data-view.org/.

NGlycositeAtlas portal

N-Linked glycosylation is one of the most abundant protein modifications and is highly relevant to disease progression in cancer.[57] With the advances in experimental and computational approaches, glycoproteomics has provided comprehensive characterization of glycosite-specific glycosylation of glycoproteins and valuable insights into their biological functions in cancer.[58–62] However, there is still a lack of the integration of large-scale characterization of glycoproteomic data from different cancer types for pan-cancer research. We identified intact N-linked glycopeptides (see Data S1) to create a database resource termed N-GlycositeAtlas 2.0, which contains more than 90,629 intact N-linked glycopeptides (representing 5,665 N-linked glycosite-containing peptides) of over 2,000 glycoproteins from CPTAC

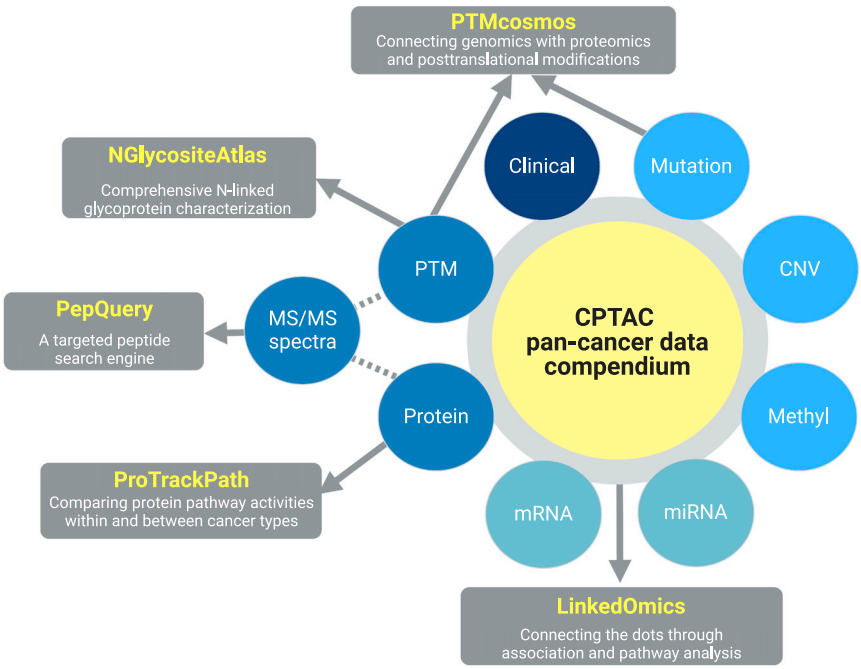


Figure 4. Web portals to CPTAC data
Multiple websites present CPTAC's proteogenomic data for visual exploration.

data. The NGlycositeAtlas database and consensus MS/MS spectra are available at https://www.biomarkercenter.org/nglycositeatlas.

ANALYTICAL CHALLENGES FOR PAN-CANCER MULTI-OMICS

With the rapid development of molecular measurement technologies, cancer datasets have become multi-modal. CPTAC has created rich proteogenomic datasets that measure DNA, RNA, and protein molecules within tumors and adjacent normal tissues (NATs). This diversity of data catalogs a comprehensive map of cellular state, providing researchers the opportunity to understand the subtle regulatory interplay between DNA mutation events that give rise to dysregulated signaling networks and the ultimate cellular phenotype. This large and comprehensive dataset presents several challenges in data integration and interpretation. In this section, we outline several important considerations for the re-use and re-analysis of proteogenomic data.

The first challenge in a proteogenomic dataset is to ensure that identifiers are harmonized. The following examples demonstrate the challenge. Many genes have multiple protein isoforms due to alternative splicing, including a noted change in splicing patterns in cancers.[63–65] Each isoform may have a unique function and combining all data into a single "gene level" measurement could obscure these differences. Suppose that mRNA data identifies two distinct transcripts. The transcriptomics data table, therefore, reports two database identifiers each with a separate quantitative value. If the proteomics data do not identify peptides that differentiate the two isoforms, which protein identifier should be used? To which transcript data should the protein abundance be compared? As orthogonal data types, proteomics and transcriptomics frequently identify different isoforms. This situation is equally complex when integrating PTMs, mutations, or epigenetics. If a phosphorylation or a coding mutation is observed, which protein isoform should it be associated with? Which transcript/protein should be used in comparison with methylation data? Mapping PTMs and coding mutations to different protein isoforms will make it difficult to study the impact of somatic mutations on PTMs. Thus, for a large multi-omics harmonization task such as presented here, we recommend careful consideration and transparency in reporting analytical methods. As potential solutions to mitigate the aforementioned challenges, we suggest the following: (1) using the same versions of genome assembly and gene annotation for the processing of data from all omics platforms and all cancer types; (2) reporting gene-level quantification when isoform level analysis is unrealistic; and (3) applying a consistent and transparent rule for representative isoform selection when representative isoform selection is needed but the data are isoform agnostic, e.g., phosphosite localization annotation.

A second challenge is embracing the full proteogenomic landscape as the molecular characterization of cells and tissues becomes more complete. We emphasize that each data type provides unique value and helps to clarify complex phenotypes. For example, the proteome and the transcriptome are distinct, and each provides a meaningful view of cellular processes. A rich body of research demonstrates that the mRNA and protein abundances frequently have a poorer correlation than expected,[66–70] a consequence of both translational and post-translational regulation.[71–74] As cancers are often characterized by regulatory dysfunction, exploring the source of this dysfunction can be best understood by combining transcriptomics and proteomics.[75] Similarly, the consequence of somatic mutation in kinases is best observed by combining genomics and phosphoproteomics. Indeed, many biological hypotheses can be best addressed by a fruitful combination of data types. To understand the consequence of genomic copy number variation, Gonçalves et al., combined genomics and proteomics and discovered widespread post-transcriptional attenuation in protein abundance mitigating the impact of gene amplification, especially to preserve stoichiometry in protein complexes.[34] The search for novel amino acid variants[76] and cancer neo-antigens[77–79] is inherently a proteogenomic investigation, as is the discovery of tumor-specific splice isoforms[80,81] and fusion proteins.[82] Combining all the proteogenomics levels into a single analysis is challenging, but the non-negative matrix factorization (NMF) methodology is frequently used for integrative clustering to highlight the unique contribution of each data type.[83]

Despite the great effort to harmonize the multi-omics datasets across different cancer studies, we want to emphasize that "batch" effects between different cancer types could still remain in the pan-cancer datasets due to both technical factors, as omics experiments of different cancer types were carried out by different labs and/or using different platforms, and biological factors, as different organs and cancer types have intrinsically different biology. Thus, when analyzing the pan-cancer data, one needs to carefully adjust for these batch effects across different cancer types. For example, when fitting a regression model to study the dependence of molecular abundances on other attributes, one can include cancer-type indicators as covariates to account for cancer-type specific mean values of molecules. Other analysis techniques, such as meta-analysis framework, could also be used to perform pan-cancer level inferences.

Finally, we focus on a challenge specific to PTMs. In the CPTAC data, we report quantitative measurement of phosphorylation and selected datasets also have data for acetylation and glycosylation. Although missing values are a regular part of all omics data, they are more pronounced in PTM data. One place where this is particularly problematic is pan-cancer analysis. If a PTM site is well quantified in one cancer type (e.g., EGFR tyrosine 1172), it may have many missing values in another, which would complicate a pan-cancer comparison of protein activation. One might be tempted to roll together all PTMs in a protein into a single measurement - e.g., the average phosphorylation state of EGFR. However, we advise against this, as PTMs at each site in a protein can be functionally independent and may not correlate across samples. Both experimental and computational approaches are being developed to improve PTM peptide identification, which will help alleviate the missing value problem in PTM proteomics.[84]

Conclusion

Pan-cancer proteogenomic data analysis requires a consistent dataset processed with a unified pipeline across all samples. Several groups have created proteogenomic datasets on cancer cohorts, exploring diverse genetic backgrounds for common cancers,[85–88] pediatric tumors,[51] or understudied tumor types.[89,90] For pan-cancer analyses it is important that individual datasets follow similar SOPs and process data in a consistent manner. Therefore, we have re-processed the data from CPTAC's 10 cancer cohorts to create a pan-cancer proteogenomic dataset. We presented the description of methods used to create this data compendium, methods of data access, as well as key considerations for pan-cancer multi-omics data analysis. This resource has been used within CPTAC for biological discoveries under various themes. We hope this also serves as a resource for the broader cancer research community to advance cancer diagnosis and treatment.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2023.06.009.

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DECLARATION OF INTERESTS

F.A. is an inventor on a patent application related to SignatureAnalyzer-GPU filed by the Broad Institute and is an employee and shareholder of Illumina Inc. since 8 November 2021.

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