Sex Differences in the Cancer Proteome

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Abstract

Proteins play a central role in cancer biology, as are the most common drug targets and biomarkers. Sex influences the proteome in many diseases, ranging from neurological to cardiovascular. In cancer, sex is associated with incidence, progression and therapeutic response, as well as characteristics of the tumour genome and transcriptome. The extent to which sex differences impact the cancer proteome remains largely unknown. To fill this gap, we quantified sex differences across 1,590 proteomes from eight cancer types, identifying 39 genes with sex-differential proteins abundance in adenocarcinomas of the lung and 15 genes in hepatocellular carcinomas, clear cell cancers of the kidney and adenocarcinomas of the pancreas. A subset of these protein differences could be rationalized by sex-differential copy number aberrations. Pathway analysis showed that sex-differential proteins in lung adenocarcinoma were enriched in MYC and E2F target pathways. These findings highlight the modest impact of sex on the cancer proteome, but the very limited power of existing proteomics cohorts for these analyses.

Introduction

Proteins play a central role in cancer biology as drug targets and as diagnostic, prognostic and predictive biomarkers – largely quantified *via* immunohistochemistry[1]. Proteomics enables high-throughput profiling protein abundance, structure and modifications, directly quantifying the terminal products of gene expression[2]. The proteome is highly dynamic and is shaped by both genetic and environmental factors. Understanding what determines proteomic characteristics is critical for identifying reliable biomarkers for precision oncology, and in mechanistic studies.

Sex influences gene expression in normal cells. For example, proteomic studies identified thousands of proteins with sex-differential abundance in brain and cerebrospinal fluid, many of which are associated with psychiatric and neurodegenerative conditions[2,3]. Blood proteomic studies revealed substantial sex differences in protein abundance associated with cardiovascular disease and obesity[4,5]. These findings highlight the importance of considering sex as a factor in proteomic studies, and yet the impact of sex on the cancer proteome remains largely unknown.

Indeed sex has well-documented effects on cancer, with extensive research characterizing sex differences at the genomic level. For example, tumours arising in male patients exhibit higher somatic mutation burdens in cancers such as bladder, melanoma, kidney and liver but lower in glioblastoma[6]. Copy number aberrations (CNAs) differ by sex and are associated with clinical phenotypes[7]. In colon cancer, genes with sex-differential essentiality have been identified[8], while in pediatric cancers, moderate sex differences in somatic mutations and methylation have been reported (Oh *et al.*, biorxiv). These findings suggest sex-specific somatic mutations may at least partially explain sex differences in cancer incidence, progression, survival and treatment response[9–15]. While sex differences at the genomic level are well studied, how these differences translate into gene expression, particularly at the protein level, is less understood. al cancer behavior and therapeutic efficacy between men and women.

To fill this gap, we analyzed 1,590 high-resolution mass spectrometry-derived proteomes from 934 cancer patients representing eight cancer types. We quantified sex differences in protein abundance in each cancer type using univariable and multivariable statistical modeling, adjusting for key confounders like age and stage. We identified sex differences in protein abundances in four cancer types, with distinct biological pathways influenced by sex. These findings underscore the importance of understanding the influence of sex on cancer molecular profiles, as it can inform patient stratification and guide the development of more effective, tailored treatments.

Results

We analyzed 1,590 proteomes across eight cancer types from CPTAC (the Clinical Proteomic Tumour Analysis Consortium): 915 tumours and 675 normal adjacent tissues (NATs; **Figure 1A**). Cancer types, abbreviations and sample numbers are in **Supplementary Table 1**. The number of samples analyzed ranged from 109 in glioblastoma (GBM) to 314 in liver hepatocellular carcinoma (LIHC)[16–23]. There were 73 ± 21 male and 34 ± 20 female patients per cancer type (median ± MAD; mean absolute deviation). On average, 8,890 ± 637 proteins were detected and quantified per patient. We applied an established multi-stage statistical approach to assess sex differences in protein abundances[6,7,24,25]. Briefly, we first excluded genes with sex differences in normal tissues, followed by a univariate analysis to identify putative sex-differential proteins and finally a multivariable analysis to control for confounding variables including age and stage (**Supplementary Table 1**).

We first analyzed sex differences in protein abundance in NATs, finding that most proteins (100 ± 0.01%) did not exhibit significant sex-differential abundance (q-value > 0.05; **Figure 1A**). To assess sex differences in protein abundance of autosomal genes in tumours, we first employed a univariable approach across the eight cancer types. We observed pronounced rightward skews in p-value distributions for kidney renal clear cell carcinoma (KIRC), LIHC, lung adenocarcinoma (LUAD) and pancreatic adenocarcinoma (PAAD), indicating strong sex differences in these cancers (**Figure 1B**; **Supplementary Figure 1**). Moderate but clear rightward skews were observed for head and neck squamous cell carcinoma (HNSC) and lung squamous cell carcinoma (LUSC; **Figure 1B**; **Supplementary Figure 1**).

Putative sex-differential proteins (q-value < 0.1) were further adjusted using multivariable linear regression (MLR) for confounders known to impact gene expression[24,25], including age, race, smoking, BMI, tumour stage and grade. In LUAD, 1,043 proteins remained significant (q-value < 0.1), while 20 proteins were identified across LUSC, GBM, PAAD, KIRC, and LIHC, with each

cohort yielding 1 to 9 significant (q-value < 0.1) proteins. No proteins remained significant after MLR adjustment in HNSC and COAD (**Figure 1A**; **Supplementary Figure 1**; **Supplementary Table 2**). Power analyses estimated that at least 187 and 181 samples are needed to detect 20 sex-differential genes (effect size range: > 0.30 or < -0.20 for HNSC and > 0.13 or < -0.12 for COAD) in these cohorts (**Supplementary Figure 2**). Thus while our analysis exploits the largest available proteomics cohorts to-date, our statistical power is limited to only large effect-sizes.

To address the limited statistical power of proteome-wide analysis, we focused on genes with sex-differential CNAs to assess their influence on sex-differential protein abundance (**Figure 1C**). Using data from the PCAWG (Pan-Cancer Analysis of Whole Genomes) project, we identified 1,939 ± 2,100 genes with putative sex-differential CNAs within each of the eight cancer types (proportion test p-value < 0.1 without adjusting for confounders; **Figure 1A**; **Supplementary Table 3**). Given the high correlation among CNAs across the genome, a strict multiple-testing correction was not applied at this stage to avoid excluding biologically relevant signals. We integrated CNA findings with proteomic data by comparing genes with sex-differential CNAs within each cancer type to those identified through MLR analysis of sex-differential protein abundance. In PAAD, KIRC, LIHC and LUAD, multiple genes with putative sex-differential CNAs also showed significant (q-value < 0.1) sex-differential protein abundance. In contrast, no sex-differential CNAs showed significant (q-value < 0.1) sex-differential protein abundance in colon adenocarcinoma (COAD), GBM, HNSC or LUSC (**Supplementary Table 2**).

Fifteen genes with putative sex-differential ploidy change and protein abundance were identified using multivariable linear regression in PAAD, KIRC or LIHC, with no overlap across cancer types (**Figure 1D**; **Supplementary Table 2**). *RAB42*, a member of the RAS oncogene family, had elevated abundance in tumours derived from male patients with KIRC tumours (q-value = 8.2×10^{-5}, effect size = 1.02). *CCNA2*, part of the cyclin family and overexpressed in multiple cancer types[26], showed higher protein abundance in males with LIHC (q-value = 2.7×10^{-4}, effect size = -

0.73). *CAST*, encoding calpain and prognostic in pancreatic cancer[26], had elevated protein abundance in males with PAAD (q-value = 2.0×10^{-3}, effect size = 0.14).

LUAD tumours had the greatest number of genes with sex-differential protein abundance, substantially more than any other cancer: 1,122 total genes ($q < 0.1$; **Figure 1B**; **Supplementary Figure 1**). Of these, only 39 showed sex-differential ploidy – thus the majority of sex differences in LUAD were not driven by CNAs (**Figure 1E**; **Figure 2A**), none of which overlap with genes significant in PAAD, KIRC or LIHC. Five ribosomal proteins (*i.e.*, *RPS5*, *RPS7*, *RPL4*, *RPL5* and *RPL18A*) had elevated expression in male LUAD tumours (q-values between 8.3×10^{-3} and 0.048, effect size between 0.11 and 0.23; **Supplementary Table 2**). *PFKP*, a key regulator of glycolysis and metabolic reprogramming in lung cancer[27], showed higher protein abundance in males (q-value = 0.024, effect size = 0.31), highlighting sex-specific differences in metabolic pathways. *CYP4F3*, implicated in drug metabolism and lung cancer[28], also had increased abundance in males (q-value = 0.01, effect size = 0.59), pointing to sex-specific roles in xenobiotic metabolism. Conversely, *THSD4*, associated with immune checkpoint inhibitor sensitivity in non-small cell lung cancer, had higher protein abundance in females (q-value = 0.011, effect size = -0.37), reflecting potential sex-dependent immune microenvironment dynamics. These findings suggest distinct biological processes, including protein synthesis, metabolism and immune modulation, influenced by sex in LUAD.

To evaluate the consistency of sex-differential protein abundance across cancer types, we examined the correlation of effect sizes between each pair of cancer types (**Supplementary Figure 3**). Overall, effect sizes showed weak to moderate correlations. Although sex-differential protein abundance was identified in PAAD, KIRC, LIHC and LUAD, little correlation was observed with other cancer types. GBM and LUSC, which showed no significant sex-differential protein abundance, exhibited the largest positive correlations, suggesting shared regulatory mechanisms influencing sex-differential protein abundance (Spearman's Rho = 0.2, p-value = 5.1×10^{-88}).

These results indicate that sex-differential proteins and the magnitude of differences vary substantially between cancer types.

Given the large number of genes with significant sex-differential protein abundance in LUAD, we next investigated whether these differences were mirrored at the RNA level. We analyzed the transcriptome data from the CPTAC-LUAD project and found a correlation between RNA and protein abundance for the 39 genes with sex-differential protein abundance, as reported above (**Supplementary Figure 4**). Cancer hallmark enrichment analysis showed these genes are significantly enriched in MYC targets (q-value = 5.0×10^{-42}), E2F targets (q-value = 3.7×10^{-26}) and G2M checkpoint (q-value = 1.0×10^{-24}; **Figure 2B**).

To understand impact of sex-difference in genomic alterations on the cancer proteome, we performed integrated analysis with CNA alterations. Previously, we reported sex differences in CNAs in both coding and noncoding regions of the genome at the pan-cancer level and in specific cancer types, including KIRC and LIHC[6,7]. We applied the Mann-Whitney U test to compare the effect sizes of sex differences in protein abundance between genes with and without sex-differential CNAs. In LUAD, KIRC, PAAD and LIHC, the cancer types where sex-differential proteins were identified, genes with sex-differential CNA status tended to have lower effect sizes in protein abundance, indicating that their protein levels were generally higher in female tumors (p-value from 3.4×10-4 to 0.023; **Figure 2C-E**). In contrast, for the other cancer types, no significant enrichment of sex-differential protein abundance was observed among genes with sex-differential CNAs, suggesting that additional regulatory mechanisms contribute to these differences (**Supplementary Figure 5**).

Discussion

Our analysis of cancer proteomes revealed variable sex differences in protein abundance across specific cancer types. Pronounced differences were observed in LUAD, while KIRC, PAAD and LIHC showed moderate variability. These sex differences can be driven by interactions between sex hormones (*e.g.*, estrogen and androgen), environmental exposures and the tumour microenvironment, which together shape the sex-dependent evolution of cancers. For example, several P450 enzymes, involved in the metabolism of tobacco-derived carcinogens, are regulated by sex hormones and may further contribute to carcinogenesis[29]. Additionally, estrogen and progestin are known to promote angiogenesis in lung cancer[30], potentially altering the tumour microenvironment to facilitate tumour growth and suppress immune surveillance. X-inactivation escape in certain X-linked genes may also lead to imbalanced gene expression of autosomal genes between males and females through regulatory mechanisms[31]. These factors all contribute to sex differences in protein abundance, ultimately affecting disease progression. Interestingly, the male-dominant lung cancer subtype, LUSC, exhibited minimal sex-related differences in the proteome, suggesting distinct tumour origins and progression pathways in LUAD and LUSC. This highlights the differential influence of endogenous and exogenous factors on tumor evolution and gene expression, leading to varying disease manifestations between sexes.

Interpreting sex differences in proteomics is inherently complex due to clinico-epidemiological variables that are partially correlated with sex or influenced by gender-associated behaviors. We applied a statistical approach combining univariable analyses and multivariable regression to identify sex-biased molecular variables while adjusting for confounders such as age, race, stage, tumour site, smoking and BMI[6,7]. However, the impact of unmeasured factors, including alcohol consumption, diet, exercise and environmental exposures, remains a significant limitation. Ideally, sex differences of tumor molecular signatures would be directly quantified using multivariable models with comparisons of their estimates and confidence intervals. However, the limited sample

sizes in current cancer proteogenomic cohorts constrain the ability to derive precise estimates, particularly given the numerous confounders. This necessitated a multi-stage design, which balances statistical power while accounting for confounders. Despite these challenges, our approach provides a critical foundation for investigating sex differences in proteomics.

This study extends our understanding of sex differences from genomics into proteomics but is limited by the number of patients and cancer types analyzed. To detect 20 sex-differential proteins with q-value < 0.05 in LUSC, at least 211 patients would be required based on an effect size threshold of > 1.86 or < -0.22 (**Supplementary Figure 2**). Larger cohorts covering a wider range of cancer types are essential for a more complete picture. Additionally, the datasets we used had imbalanced sample sizes, with fewer female patients in most cancers. Future studies should aim for sex-balanced patient cohorts that reflect the sex-specific prevalence of specific cancer types, ensuring unbiased representation and improving the generalizability of findings. Increasing the sample size is also critical for studying sex differences in low-frequency somatic mutations and their impact on the proteome. To improve statistical power, meta-analysis across independent datasets can be leveraged and statistical methods can help adjust for sample imbalance. Integrating clinical and epidemiological data, including hormone levels, environmental exposures and lifestyle factors, will be critical for understanding the drivers of sex-differential proteomics. While collecting comprehensive metadata is challenging, wearable devices could provide continuous, unbiased tracking of lifestyle variables. Additionally, large-scale biobank data can be leveraged to disentangle the interactions between sex, confounders and tumour proteome alterations. Overall, our findings highlight the importance of investigating sex as a key factor in cancer biology and treatment response, paving the way for personalized therapies based on sex-specific molecular profiles.

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Author Contributions

Initiated Study: PCB, CZ, JO, NZ, CHL

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Methods

Proteomics and Transcriptomics Data

Protein abundance matrices from eight non-reproductive cancer cohorts (HNSC, LUAD, GBM, KIRC, LUSC, COAD, LIHC and PAAD) were obtained from the Clinical Proteomic Tumor Analysis Consortium (CPTAC)[16,17,19–22]. The processed and normalized protein abundance matrices of tumour and normal adjacent tissues (NATs), along with associated clinical information, were downloaded from Proteomic Data Commons (PDC, https://proteomic.datacommons.cancer.gov). Eight samples were excluded from analysis due to failing quality control, either showing high correlation (Pearson's R > 0.9) or being female samples with high Y-chromosomal protein abundance. Genes allocated on the sex chromosomes are excluded from analysis as they are sex-biased by nature.

RNA abundance data (FPKM) from CPTAC's LUAD cohort were retrieved using the R Package TCGAbiolinks (v3.16).

Sex Difference of Copy Number Aberrations

Sex difference of copy number aberrations (CNAs) for the eight cancer types were estimated as previously described[6]. PCAWG consensus CNAs (syn8042988) were categorized into gain, neutral, or loss calls per gene. Putative sex-differential genes were identified by comparing the proportions of tumours with gains and losses between sexes using proportion tests. No FDR correction was applied at this exploratory stage and genes with a p-value < 0.1 were selected for further analysis.

Sex difference of Protein Abundance

A multi-step statistical analysis workflow was used to identify genes with sex-differential protein abundance (**Figure 1B**). First, t-tests were performed on NAT protein abundance to evaluate sex differences, followed by FDR correction (q-value < 0.05). Next, t-tests were applied to tumour

samples to assess sex-differential protein abundance, with FDR correction applied (q-value < 0.1). Genes meeting this threshold were further adjusted using multivariable linear regression (MLR) to control for confounders, including age, tumour stage, race and smoking history[6,7], with FDR correction (**Supplementary Table 1**). MLR results were then annotated with sex-differential CNA status. Cancer hallmark enrichment analysis was performed using the R package hypeR (1.10.0)[32] and MSigDB gene sets[33,34] for genes with q-value < 0.1. Mann-Whitney's test was used to compare the effect sizes of sex differences in protein abundance between genes with and without sex-differential CNAs.

Sex Differences in RNA Abundance in LUAD

The 39 genes with significant sex-differential protein abundance (q-value < 0.1, MLR analysis) were selected and the corresponding RNA abundances were extracted from the TPM matrix. Multivariable linear regression models were applied to each gene of the tumour samples adjusting confounding variables, including age, tumour stage and smoking history, with FDR correction.

Power Analysis

Power analysis was conducted per protein for each cancer type, using the effect size and standard error (SE) calculated from t-tests, under the assumption of equal standard errors between males and females and equal numbers of male and female patients. To focus on biologically meaningful differences, we restricted the analysis to the top 20% of genes ranked by absolute effect size in each cancer type. The t-value for each protein was calculated as:

$$t_{ij} = \frac{Effect\ Size}{SE\ \times \frac{\sqrt{n}}{\sqrt{j}}}$$

where n represents the total number of samples with cancer type i analyzed in the study, and j is the sample size of interest. For each sample size j, the t values for all proteins were converted into p-values using the degree of freedom $j - 1$, assuming a two-tailed test. These p-values were

then adjusted to q-values using the FDR method. The analysis was iteratively repeated across varying sample sizes to determine the number of proteins identified with significant sex differences (q-value < 0.05) at each sample size.

Statistical Reporting

Results are reported as median values followed by the median absolute deviation (MAD), unless otherwise specified.

Data Availability

Protein abundance matrices were downloaded from Proteomic Data Commons (PDC) with the following accession numbers: PDC000221 (HNSC), PDC000153 (LUAD), PDC000204(GBM), PDC000127(KIRC), PDC000234 (LUSC), PDC000109 (COAD), PDC000198 (LIHC) and PDC000270 (PAAD). RNA-seq data for LUAD are available on Genomic Data Commons (GDC, Project: CPTAC-3, Primary Site: Lung, Primary Diagnosis: Adenocarcinoma, NOS). Data used in this publication were generated by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (CPTAC).

Code Availability

All statistical analysis and data visualization were performed in the R statistical environment (v4.0.2). All visualization were performed using the BoutrosLab.plotting.general package (v6.0.3).[35,36] Code is available at https://github.com/uclahs-cds/project-CancerBiology-SexDifferencesv3PedProtFunc.

Figure Legends

Figure 1: Cancer-type variability in proteomic sex differences

A) Overview of sex difference in cancer proteome across eight cancer types: lung adenocarcinoma (LUAD), hepatocellular carcinoma (LIHC), clear cell renal cell carcinoma (KIRC), pancreatic and ductal adenocarcinoma (PAAD), lung small cell carcinoma (LUSC), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC) and glioblastoma (GBM). From top to bottom, the number of female (pink) and male (blue) patients in each cancer type, total number of proteins quantified and number of genes with significant protein abundant favoring males (blue) and female (pink; q-value < 0.1) in normal adjacent tissues (NATs) and tumours. **B)** P-value distribution of sex difference in protein abundance across eight cancer types: colon adenocarcinoma (COAD), glioblastoma (GBM), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), clear cell renal carcinoma (KIRC), hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD) and pancreatic ductal adenocarcinoma (PAAD). P-values were calculated using t-test. **C)** Statistical analysis workflow for identifying sex-differential proteins linked to sex-differential ploidy changes. **D)** Genes with significant sex differences in protein abundance (q-value < 0.1) in PAAD, KIRC and LIHC. Dot colour represents a favor for males (blue) and females (pink), dot size represents effect sizes, while cell background darkness indicates q-value. Statistic values were calculated using multivariable linear regression with false discovery rate correction. **E)** Relative abundance of genes with significant sex differences in protein abundance (q-value < 0.1) in LUAD. Row annotation heatmap indicates sex differences in the copy number aberrations (CNA) for each gene, with green and yellow indicating CNA gain favoring in males and females, respectively, and blue and orange indicating CNA loss.

Figure 2: Large proteomic sex differences in lung adenocarcinomas

A) Volcano plots showing effect sizes and q-values for sex difference in protein abundance from tumours derived from lung adenocarcinoma (LUAD) patients. Effect sizes and q-values were calculated using t-test with false discovery rate correction. Light blue and pink indicate a significant bias in protein abundance (q-value < 0.1) favoring males and females, respectively Dark blue and pink represent an additional biased copy number aberrations (CNA) for males and females. **B)** Top 20 cancer hallmarks enriched in genes with sex-differential protein abundance in LUAD. Dot size indicates the number of genes mapped to the each cancer hallmark, while colour denotes the q-value. **C)** Dot map of Mann-Whitney U test comparing the effect sizes of sex differences in protein abundance between genes with and without sex-differential CNAs. Dot color indicates the direction of effect size (blue for male-biased, red for female-biased), dot size represents effect size of Mann-Whitney U test, and background darkness corresponds to statistical significance (darker indicates lower p-values). **D-E)** From top to bottom: fold change (FC) in protein abundance (males - females), heatmap of protein relative abundance, sex differences of copy number gain (males - females), proportion value of copy number gain in males (blue) and females (pink) separately, sex differences of copy number loss (males – females), and proportion value of copy number loss in males (blue) and females (pink). Stacked figures are aligned according to the gene coordinates in the human genome. Stacked panels are aligned according to the gene coordinates at the human genome.

References

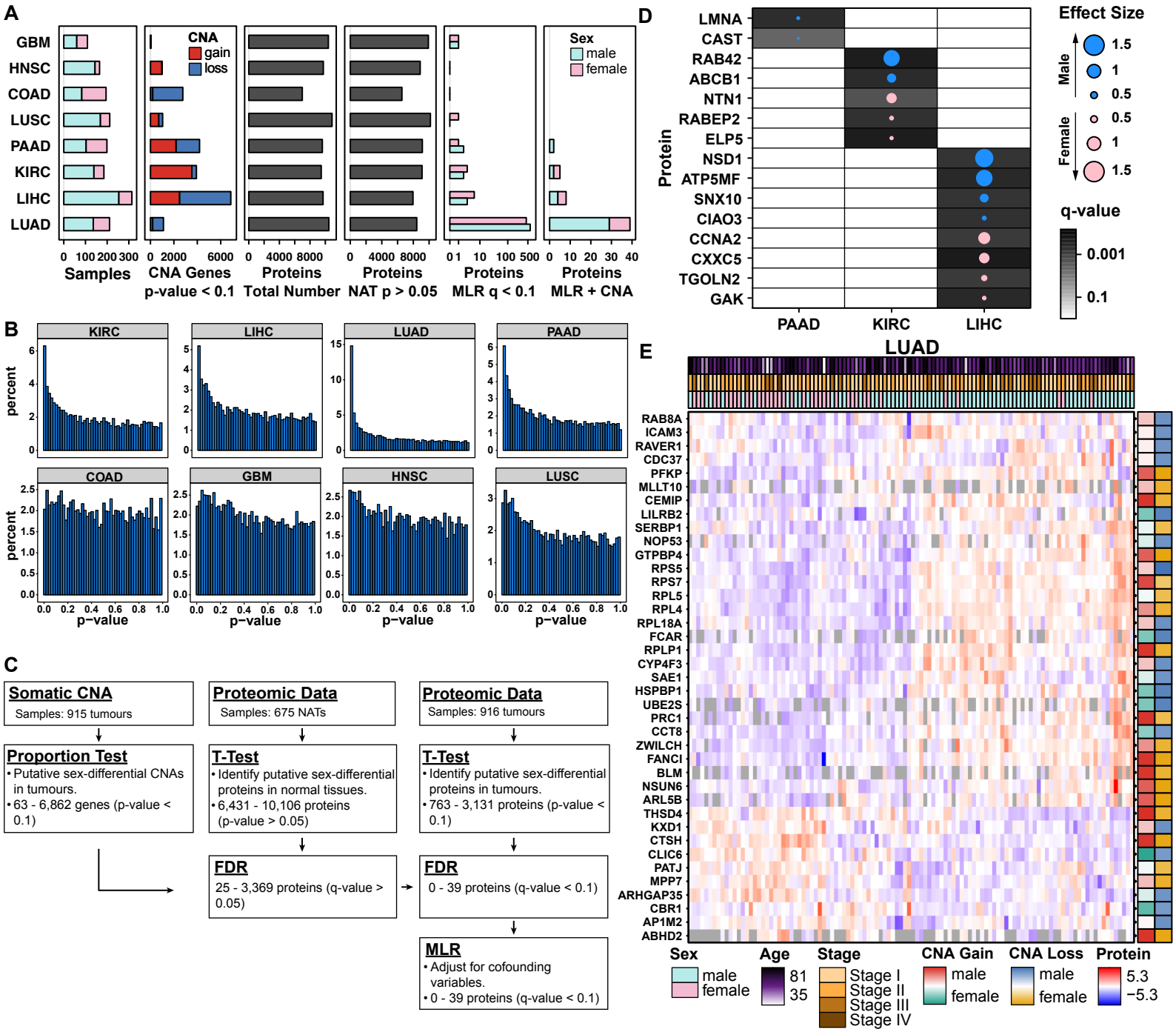
1. Passaro, A. *et al.* Cancer biomarkers: Emerging trends and clinical implications for personalized treatment. *Cell* **187**, 1617–1635 (2024).
2. Seo, D. *et al.* Sex and aging signatures of proteomics in human cerebrospinal fluid identify distinct clusters linked to neurodegeneration. *medRxiv* 2024.06.18.24309102 (2024) doi:10.1101/2024.06.18.24309102.
3. Wingo, A. P. *et al.* Sex differences in brain protein expression and disease. *Nat Med* **29**, 2224–2232 (2023).
4. Halland, H., Vitorino, R., Gerdts, E., Meyer, K. & Kararigas, G. Sex-biased proteomic regulation in obesity. *European Heart Journal* **45**, ehae666.2894 (2024).
5. Liu, Y. *et al.* Sex differences in proteomics of cardiovascular disease: results from the Yale-CMD registry. *European Heart Journal* **45**, ehae666.3091 (2024).
6. Li, C. H. *et al.* Sex differences in oncogenic mutational processes. *Nat Commun* **11**, 4330 (2020).
7. Li, C. H., Haider, S., Shiah, Y.-J., Thai, K. & Boutros, P. C. Sex Differences in Cancer Driver Genes and Biomarkers. *Cancer Res* **78**, 5527–5537 (2018).
8. Zeltser, N., Zhu, C., Oh, J., Li, C. H. & Boutros, P. C. Sex Differences in Cancer Functional Genomics: Gene Dependency and Drug Sensitivity. 2025.02.05.636540 Preprint at https://doi.org/10.1101/2025.02.05.636540 (2025).
9. Siegel, R. L., Miller, K. D., Fuchs, H. E. & Jemal, A. Cancer Statistics, 2021. *CA Cancer J Clin* **71**, 7–33 (2021).
10. Rahbari, R., Zhang, L. & Kebebew, E. Thyroid cancer gender disparity. *Future Oncol* **6**, 1771–1779 (2010).
11. Konstantinidis, I. T. Trends in Presentation and Survival for Gallbladder Cancer During a Period of More Than 4 Decades: A Single-Institution Experience. *Arch Surg* **144**, 441 (2009).

12. Wainer, Z. *et al.* Sex-Dependent Staging in Non–Small-Cell Lung Cancer; Analysis of the Effect of Sex Differences in the Eighth Edition of the Tumor, Node, Metastases Staging System. *Clin Lung Cancer* **19**, e933–e944 (2018).
13. Kammula, A. V., Schäffer, A. A., Rajagopal, P. S., Kurzrock, R. & Ruppin, E. Outcome differences by sex in oncology clinical trials. *Nat Commun* **15**, 2608 (2024).
14. Conforti, F. *et al.* Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol* **19**, 737–746 (2018).
15. Milano, G. *et al.* Influence of sex and age on fluorouracil clearance. *J Clin Oncol* **10**, 1171–1175 (1992).
16. Huang, C. *et al.* Proteogenomic insights into the biology and treatment of HPV-negative head and neck squamous cell carcinoma. *Cancer Cell* (2021) doi:10.1016/j.ccell.2020.12.007.
17. Gillette, M. A. *et al.* Proteogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma. *Cell* **182**, 200-225.e35 (2020).
18. Satpathy, S. *et al.* A proteogenomic portrait of lung squamous cell carcinoma. *Cell* **184**, 4348-4371.e40 (2021).
19. Wang, L.-B. *et al.* Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell* (2021) doi:10.1016/j.ccell.2021.01.006.
20. Clark, D. J. *et al.* Integrated Proteogenomic Characterization of Clear Cell Renal Cell Carcinoma. *Cell* **179**, 964-983.e31 (2019).
21. Vasaikar, S. *et al.* Proteogenomic Analysis of Human Colon Cancer Reveals New Therapeutic Opportunities. *Cell* **177**, 1035-1049.e19 (2019).
22. Gao, Q. *et al.* Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. *Cell* **179**, 561-577.e22 (2019).
23. Cao, L. *et al.* Proteogenomic characterization of pancreatic ductal adenocarcinoma. *Cell* **184**, 5031-5052.e26 (2021).

24. Li, C. H., Haider, S. & Boutros, P. C. Ancestry Influences on the Molecular Presentation of Tumours. 2020.08.02.233528 Preprint at https://doi.org/10.1101/2020.08.02.233528 (2020).
25. Li, C. H., Haider, S. & Boutros, P. C. Age influences on the molecular presentation of tumours. *Nat Commun* **13**, 208 (2022).
26. Uhlen, M. *et al.* Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* **28**, 1248–1250 (2010).
27. Shen, J. *et al.* PFKP is highly expressed in lung cancer and regulates glucose metabolism. *Cell Oncol (Dordr)* **43**, 617–629 (2020).
28. Yin, J. *et al.* Pathway-analysis of published genome-wide association studies of lung cancer: A potential role for the CYP4F3 locus. *Mol Carcinog* **56**, 1663–1672 (2017).
29. Penaloza, C. G. *et al.* Sex-dependent regulation of cytochrome P450 family members Cyp1a1, Cyp2e1, and Cyp7b1 by methylation of DNA. *FASEB J* **28**, 966–977 (2014).
30. Marquez-Garban, D. C. *et al.* Progesterone and estrogen receptor expression and activity in human non-small cell lung cancer. *Steroids* **76**, 910–920 (2011).
31. San Roman, A. K. *et al.* The human Y and inactive X chromosomes similarly modulate autosomal gene expression. *Cell Genom* **4**, 100462 (2024).
32. Federico, A. & Monti, S. hypeR: an R package for geneset enrichment workflows. *Bioinformatics* **36**, 1307–1308 (2020).
33. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**, 15545–15550 (2005).
34. Liberzon, A. *et al.* Molecular signatures database (MSigDB) 3.0. *Bioinformatics* **27**, 1739–1740 (2011).
35. P'ng, C. *et al.* BPG: Seamless, automated and interactive visualization of scientific data. *BMC Bioinformatics* **20**, 42 (2019).

36. Shannon, P. *et al.* Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res.* **13**, 2498–2504 (2003).

Figure 1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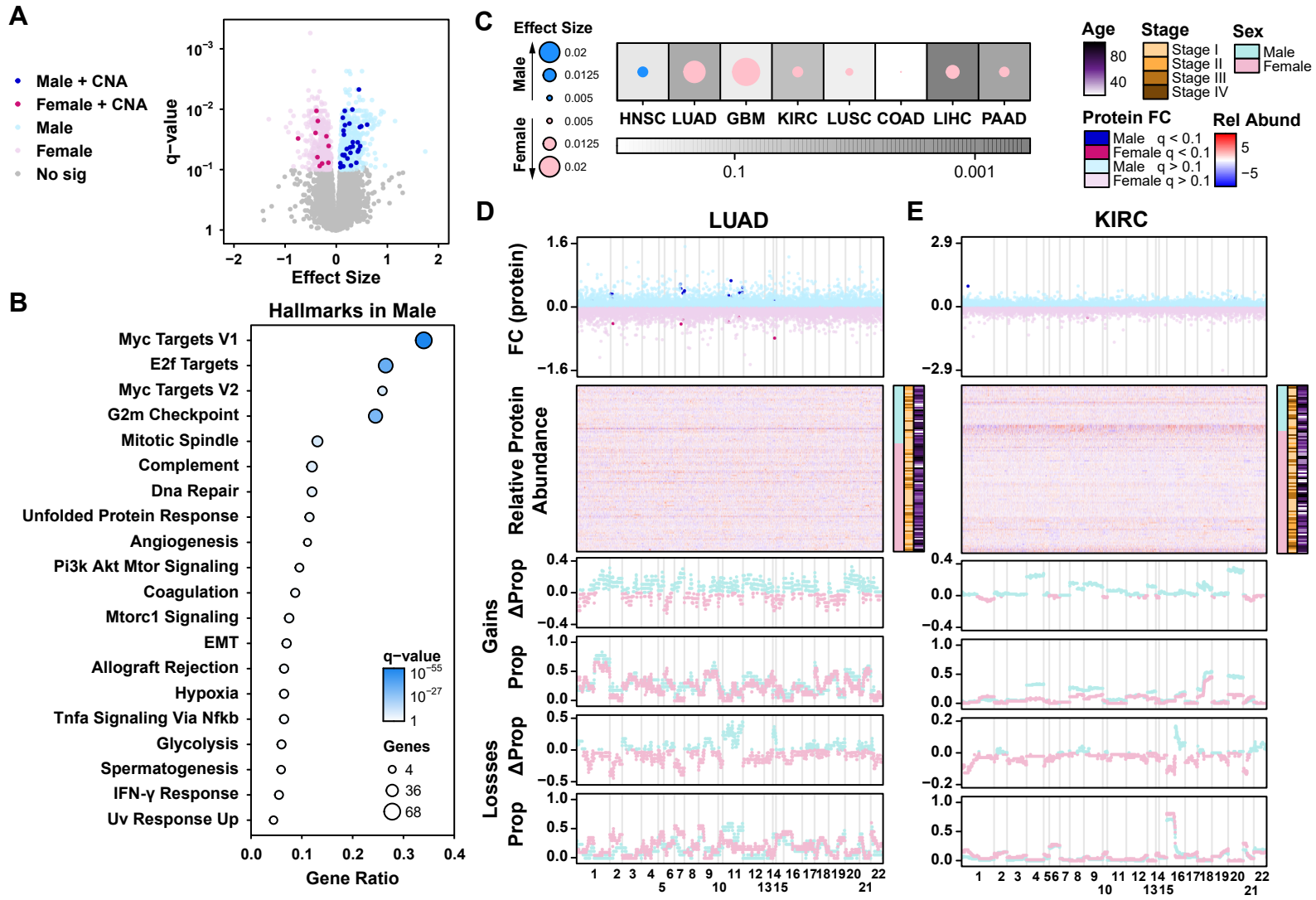
Somatic CNA
Samples: 915 tumours
Proteomic Data
Samples: 675 NATs
Proteomic Data
Samples: 916 tumours
Proportion Test
• Putative sex-differential CNAs in tumours.
• 63 - 6,862 genes (p-value < 0.1)
T-Test
• Identify putative sex-differential proteins in normal tissues.
• 6,431 - 10,106 proteins (p-value > 0.05)
T-Test
• Identify putative sex-differential proteins in tumours.
• 763 - 3,131 proteins (p-value < 0.1)
FDR
25 - 3,369 proteins (q-value > 0.05)
FDR
0 - 39 proteins (q-value < 0.1)
MLR
• Adjust for cofounding variables.
• 0 - 39 proteins (q-value < 0.1)
D
LMNA
CAST
RAB42
ABCB1
NTN1
RABEP2
ELP5
NSD1
ATP5MF
SNX10
CIAO3
CCNA2
CXXC5
TGOLN2
GAK
Protein
PAAD
KIRC
LIHC
Effect Size
Male
Female
1.5
1
0.5
q-value
0.001
0.1
E
LUAD
RAB8A
ICAM3
RAVER1
CDC37
PFKP
MLLT10
CEMIP
LILRB2
SERBP1
NOP53
GTPBP4
RPS5
RPS7
RPL5
RPL4
RPL18A
FCAR
RPLP1
CYP4F3
SAE1
HSPBP1
UBE2S
PRC1
CCT8
ZWILCH
FANCI
BLM
NSUN6
ARL5B
THSD4
KXD1
CTSH
CLIC6
PATJ
MPP7
ARHGAP35
CBR1
AP1M2
ABHD2
Sex
male
female
Age
81
35
Stage
Stage I
Stage II
Stage III
Stage IV
CNA Gain
male
female
CNA Loss
male
female
Protein
5.3
−5.3

Figure 2


A
Male + CNA
Female + CNA
Male
Female
No sig
q−value
Effect Size
B
Hallmarks in Male
Myc Targets V1
E2f Targets
Myc Targets V2
G2m Checkpoint
Mitotic Spindle
Complement
Dna Repair
Unfolded Protein Response
Angiogenesis
Pi3k Akt Mtor Signaling
Coagulation
Mtorc1 Signaling
EMT
Allograft Rejection
Hypoxia
Tnfa Signaling Via Nfkb
Glycolysis
Spermatogenesis
IFN-γ Response
Uv Response Up
q−value
Genes
Gene Ratio
C
Effect Size
Male
Female
HNSC LUAD GBM KIRC LUSC COAD LIHC PAAD
Age
Stage
Sex
Stage I
Stage II
Stage III
Stage IV
Male
Female
Protein FC
Male q < 0.1
Female q < 0.1
Male q > 0.1
Female q > 0.1
Rel Abund
D
LUAD
E
KIRC
FC (protein)
Relative Protein Abundance
Gains
Lossses
ΔProp
Prop