1. Introduction

Sex differences are evident in the incidence and mortality of diverse cancers. With the development of personalized approaches in cancer treatment, the impact of sex differences has not been systematically incorporated into preclinical and clinical cancer research. The molecular mechanisms underlying sex differences in cancer have not been elucidated. Here, we developed the first database of Sex Differences in Cancer (SDC), a web-based public database that integrates resources from multiple databases, including The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression Project (GTEx), UCSC Xena, Broad Institute Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC). SDC contains 27 types of cancers, 6 types of molecular data, more than 10,000 donors, 977 cancer cell lines were used to analyze sex differences among cancers. It provides five main modules: Survival and phenotype, Molecular differences, Signatures and pathways, Therapy response, Download. Users can download the all the visualized results and raw data after analysis. Collectively, SDC is the first integrated database to analyze sex differences in cancer on the web server, which will strengthen our understanding of the role of sex in cancers. It is implemented in Shiny-server and freely available for public use at http://sdc.anticancer.xyz.

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studies on sex effect were limited to individual genes, single molecular data types, and single cancer lineages, which lack association and are not attributed to signaling pathways. There is also a lack of exploration of sex differences in common molecular subtypes.

Therefore, building a public database is vital for collecting omics data and conducting integrative and in-depth analysis with sex differences. Here, we developed the SDC, the first web-based public database for accessing reproducible comprehensive differences analysis between the sexes that covers modules including phenotypic, molecular, and therapeutic response. These results can be quickly queried and presented in a customized manner via the website (http://sdc.anticancer.xyz), to utilize sex insights in the study of molecular oncology research and cancer therapy.

2. Methods

Most of the analysis is run in R 3.6, and the code is open-sourced on GitHub (https://github.com/longfei8533/SDC) for reference and repetition.

2.1. Data source

Biological data are mainly from TCGA, GTEx, CCLE and GDSC. Part of the data are from the collation and analysis results of UCSC Xena (http://xena.ucsc.edu/). Cancer incidence and mortality data were manually downloaded from “CANCER TODAY” page at The Global Cancer Observatory (https://gco.iarc.fr). Overall Survival (OS), Progression-Free Interval (PFI), Disease-Free Interval (DFI), and Disease-Specific Survival (DSS) were defined and calculated in previous study[21], and the supplementary data was download for further analysis. Molecular subtype data were obtained from 26 published papers collected by UCSC Xena. Tumor microenvironment (TME) subtypes data obtained from previous study[22]. Cancers with <10 samples in one sex were excluded from analysis.

2.2. Tumor purity

ESTIMATE is a method that uses gene expression profiles of 141 immune genes and 141 stromal genes to infer tumor cellularity[23]. Tumor purity was added as a covariable to the model for correction during mRNA, miRNA, and copy number variation (CNV) analysis.

2.3. Survival analysis and molecular subtypes

Survival analysis was performed with the Log-rank test. Fisher's exact test was performed to test the sex bias of different molecular subtypes.

2.4. mRNA and miRNA

The package DESeq2 [24] provides methods to test the differential expression of gene between female and male, and gives the P and log2FoldChange value based on a model using the negative binomial distribution. The tumor purity was added as a covariable to the model for correction. log2FoldChange <0 indicates that the expression of gene is male-biased; log2FoldChange >0 indicates that the expression of gene is female-biased. Tissue types in the GTEx database were converted into TCGA codes for easy comparison of sex differences between tumors and normal tissues.

The target genes of differentially expressed miRNA were predicted by TargetScanHuman [25]. Counts per million (CPM) was computed and used to normalize mRNA and miRNA data. Then, the Pearson correlation coefficient was used to analyze the correlations.

2.5. DNA methylation

The ChAMP package [26] was used for the analysis of Illumina Methylation beadarray data (TCGA 450 k). Differential methylation probes, differential methylation regions and differential methylation were shown individually.
Fig. 2. Home page function. A. Module descriptions and shortcuts are provided on the home page. Estimated age-standardized incidence and mortality rates (World) in 2020 for multiple types of cancers are displayed at the bottom of the page. B. Multiple analysis results can be quickly viewed by selecting one cancer type in the red box on the "Quick view" panel.
2.6. Somatic mutation and copy number variation

Fisher’s exact test was performed on 2×2 contingency table generated from female and male cohorts to find differentially mutated genes. The total mutation burden was defined as the number of single nucleotide variant (SNV) and INDEL in the whole genome. Significance was calculated by Wilcox rank-sum test. In gene level, the Gistic2 copy number was used to estimate copy number differences between female and male. Further, CNV burdens including broad CNV scores (BCS), focal CNV scores (FCS) and global CNV scores (GCS), which were computed by CNApp [27]. Finally, we recalculated copy number segments in the 1 M interval to obtain the region profile of CNV. Suppose there are \( N \) segments on the 1 M interval, with length \( L \) bases and copy number \( C \).

Averagecopynumber = \( \sum_{n=1}^{N} (C_n \times L_n) / \sum_{n=1}^{N} (L_n) \)

2.7. Tumor microenvironment

We used the IOBR R package [28] to decode tumor microenvironment (TME) contexture using 7 published methodologies CIERSORT [29], ESTIMATE [23], quantTseq [30], TIMER [31], IPS [32], MCPCounter [33], EPIC [34]. These methods are based on computational inference of gene expression profiles.

2.8. Signatures and pathways

255 published signature gene sets were collected by IOBR, involving tumor microenvironment, tumor metabolism, m6A, exosomes, microsatellite instability, and tertiary lymphoid structure. We calculated the signature scores for all samples in the TCGA using three methodologies of ssGSEA, PCA and Z-score.

The PARADIGM algorithm integrates pathway, expression and copy number data to infer the activation of pathway features within a superimposed pathway network structure. This dataset is ssGSEA scores for 1387 constituent pathways, then Z transformed [35].

2.9. Treatment response

Drug sensitivity data in cancer cell lines from the CCLE and GDSC were integrated, and these cancer cell lines were grouped by sex. Then, \( IC_{50} \) of the individual drug to male or female were
compared. Tumor Immune Dysfunction and Exclusion (TIDE) [36] was used to predict the immunotherapy response from the gene expression profiles of cancer tissue samples. Conserved pan-cancer microenvironment subtypes can predict response to immunotherapy [22]. Molecular profiles of cancer can be clustered into four distinct microenvironments termed (1) immune-enriched, fibrotic (IE/F); (2) immune-enriched, non-fibrotic (IE); (3) fibrotic (F); and (4) immune-depleted (D).

3. Implementation and results

3.1. The database overall design

The web was developed with R and Shiny following a modular and robust design. The data were preprocessed locally with some of the results loaded into memory for speed and another part stored in SQLite database (Fig. 1). SDC uses a series of user-friendly interfaces to display results. Because of the Bootstrap and Shiny dependency, the interfaces are dynamic and interactive on a variety of devices with different screen sizes, and five main modules were provided, including (i) Survival and phenotype, (ii) Molecular differences, (iii) Signatures and pathways, (iv) Therapy response, (v) Download. All customized resulting images can be downloaded directly in PNG or PDF format. For “Download” module, all pre-processed data can be downloaded, most of which are saved as R data, and can be quickly loaded into the R environment by the R function “readRDS”. All the raw data used for analysis is also linked for easy access.

3.2. Web interface and usage

The home page provides a module description and a quick jump (Fig. 2A). The global multi-cancer morbidity and mortality histogram is displayed at the bottom of the page, providing a quick view of the overall disease burden. The Molecular differences module (Fig. 4) allows users to explore the molecular subtypes and their expressions. The Survival and phenotype module helps users understand the survival outcomes associated with different molecular subtypes. The Molecular differences module visually compares the differential gene expression between tumor and normal tissues. The Signatures and pathways module provides insights into the biological pathways involved in cancer. The Therapy response module predicts the potential response to immunotherapy based on the molecular subtype. The Download module enables users to download all pre-processed data for further analysis.
and intuitive view of gender differences. The “Quick View” function is for the quickly selection of 1 in 27 tumor types for preview (Fig. 2B).

For “Survival and phenotype” module, users can select different age stratifications and different endpoints to study differences in survival in specific cancers (Fig. 3A). Statistical analysis was performed on clinical characteristics of cancer patients and presented in the table (Fig. 3B). Findings from Fig. S1-S4 showed that there was no significant difference in survival between females and males in lung cancer, while, in esophageal and gastric cancers, females have a better prognosis than males.

Six data categories of analysis are included in the “Molecular differences” module. (i) For molecular subtypes, fisher’s exact test results revealed sex-biased molecular subtypes (Fig. 4A). (ii) For mRNA, users can screen differentially expressed mRNAs under tumor purity correction. By inputting the minimum P value and fold change, users can further filter the highly reliable differential genes, and the results can be showed as a scatter plot (Fig. 4B). Data in mRNA level from normal GTEx tissues were calculated in the same way, so it is possible to discover which differential expression of gene are inherent before tumor development and which are caused after tumor development. Meanwhile, the results are displayed in the “Venn” panel (Fig. 4C). The detailed description of all genes can be jumped to GeneCard (https://www.gene-cards.org/) with the aid of the hyperlink. (iii) miRNAs are filtered and presented in the same way. The score for target gene prediction of miRNA is shown in the table in the “Target” panel, and the correlation between genes and miRNAs can be further viewed on the “Correlation” panel (Fig. 4D). (iv) Differential DNA methylation in three levels, including CpG island, methylation regions and methylation blocks, were shown in the scatter plot and table. (v) Somatic mutation (SNV and INDEL) results that mainly include differences in mutation frequency of gene and total tumor mutation burden. (vi) For CNV, the Gistic2 copy number was used to estimated copy number differences between females and males in gene level. And, CNV burdens including BCS, FCS and GCS, which has been reported to be potential biomarkers for prognosis and immunotherapy [37,38], were calculated with purity correction (Fig. 4E). Further, we recalculated copy number segments (log2(tumor/normal)) in the 1 M interval to obtain the region profile of CNV (Fig. 4F).

For “Signatures and pathways” module, “Tumor microenvironment”, “Signatures” and “Pathways” three functions can be used. (i) Due to the importance of tumor-infiltrating immune cells in cancer treatment efficacy and patient prognosis [39], score for the tumor-infiltrating immune cells was calculated using 7 pub-

Fig. 5. Signatures and Pathways module. A. Analysis of differences in the tumor microenvironment. (i) is to select one of the seven published methods. (ii) can convert whether the plot is displayed as a p-value or asterisks. B. Analysis of the signal signature difference. (i) indicates that tumor-related signatures can be added and removed for presentation on the same plot. (ii) is three methods for signature calculation. (iii) is the two buttons to display the signature source and genes within the signature.
lished methods [23,29–34]. Different analysis was then performed based on the score and users can choose to display the exact P value on the box plot or a more intuitive symbol (Fig. 5A). (ii) In the “Signatures” panel, 255 tumor-associated signatures scores were calculated using ssGSEA, PCA and Z-score methods and compared between the sexes. Users can click on the two buttons at the bottom of the page to find the original article and signature genes (Fig. 5B). (iii) In the “Pathways” panel, different analysis was performed on pathway activity scores of 1387 constituent PARADIGM pathways.

For “Therapy response” module, “Chemotherapy response” and “Immunotherapy response” panels were included. In the “Chemotherapy response” panel, users can select one of three databases and then select a drug or target to explore the sensitivity of tumor cell lines to the drug. The results were presented as a box plot and volcano plot (Fig. 6A). In the “Immunotherapy response” panel, patients with a TIDE score <0 were defined as positive responders to immunotherapy (Fig. 6B). Patients with IE and IE/F subtypes are more likely to benefit from immunotherapy, when using tumor microenvironment subtypes to predict immunotherapy response.

4. Future developments and discussion

As we have found that there were significant phenotypic differences between the two sexes that were disruptive to causal inferences, more covariates will be added to the model in the future or pre-treat the population with propensity score matching, so that to obtain a better and unbiased estimation. Our next plan is to integrate more samples and more types of biological data (e.g. immunotherapy patients, proteomics and epigenetics data) and mine relationships between modules (As shown in Fig. 1), the purple line represents the possible association to provide more comprehensive and accurate insights. In the coming future, users’ data uploading function and analysis service will be provided to assess gender bias and generate reports.

Overall, SDC is the first comprehensive and user-friendly web database to study molecular differences, pathway differences, and therapeutic response differences between males and females in cancer. Based on this web database, we aim to integrate more comprehensive data and provide more evidence to explain the sex differences in cancer systematically, so that to facilitate antitu-
Declarations of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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