CellKb Immune: a manually curated database of mouse hematopoietic marker gene sets optimized for rapid cell type identification

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Abstract

Single-cell RNA-seq is widely used to study transcriptional patterns of genes in individual cells. In spite of current advances in technology, assigning cell types in single-cell datasets remains a bottleneck due to the lack of a comprehensive reference database and a fast search method in a single tool. CellKb Immune is a knowledgebase of manually collected, curated and annotated marker gene sets from cell types in the mouse immune response. It finds matching cell types in literature given a list of genes using a rank-based approach optimized for rapid searching across marker gene lists of differing lengths. We evaluated the contents and search methodology of CellKb Immune using a leave-one-out approach. We further used CellKb Immune to annotate previously defined marker gene sets from Immgen to confirm its accuracy and coverage. CellKb Immune provides an easy-to-use database with a fast and reliable method to find matching cell types and annotate cells in single-cell experiments in a single tool. It is available at https://www.cellkb.com/immune.

1. Introduction

Using single-cell RNA sequencing, it is now possible to get the detailed gene expression pattern in each cell for millions of cells in a single experiment under varying conditions[1]. Annotating cells by assigning a type or state to them is important for correct interpretation of results. This requires a resource that contains a comprehensive reference database of cell types and marker gene sets from literature along with a search method that allows users to look for matching cell types. On the one hand, various computational tools have been developed for the identification of matching cell types given a reference database and a gene list[2,3]. On the other hand, several single cell reference datasets or atlases have been prepared[1,4,5] or compiled into aggregate reference databases of cell types and marker gene sets[6–9]. However, it takes considerable effort from the user to download the reference database/dataset and programmatically integrate a search method with it for cell type annotation.

CellKb Immune addresses all these issues by collecting and aggregating mouse immune-related cell type signatures from literature and providing an online interface to find matching annotated cell types easily, quickly and accurately.

2. Materials and Methods

Marker gene sets are selected from published experimental studies if they satisfy the following criteria: 1) Deposition of raw data in public databases, 2) Availability of data for download, 3) Description of experimental method used, 4) Availability of number of cells studied, 5) Description of computational methods used to normalize, filter and cluster cell types, along with identification of cluster-specific genes, 6) Availability of associated values (e.g. average expression, fold change, statistical significance), 7) Availability of sufficient number of valid gene identifiers in the marker gene set as mapped to the latest version of the Ensembl database[11].

Marker gene sets are extensively curated to include valid genes and cell types only. Cell types, tissue names and disease conditions are assigned standardized ontology terms[12–14]. Associated values given by authors with each signature are also stored in CellKb Immune. These include, but are not limited to, rank, score, average expression, log fold change and corrected/uncorrected p-values. The cell type specific marker genes are either directly taken as defined by the authors, or they are calculated based on the associated values provided. Each cell type is assigned a reliability score that gives an indication of how similar it is to other cell types of the same ontology. Finally, all marker genes and their annotations are stored in a uniform format that enables rapid search and retrieval of the data.

Query genes given by the user are compared with every marker gene set in the database and a match score is computed, based on the number of common genes between the query and cell type, their ranks, their rank differences and the total number of significant genes in the cell type. This results in cell types sharing highly ranked genes with the query being assigned higher match scores. The match score also accounts for differences in the sizes of gene lists between the query and various cell types, such that cell types with fewer significant genes are not disregarded.

3. Results

3.1 Database content

CellKb Immune contains author-defined immune cell type marker gene sets manually collected from publications describing mainly single-cell, and selected bulk RNA-seq or microarray experiments[2,10] in *Mus musculus*.

CellKb Immune contains:

- 2,033 marker gene sets from mouse.
- 205 unique annotated hematopoietic cell types (Cell Ontology CL:0000988 and all its descendants).
- 186 publications selected from approximately 10,000 studies published between 2013 and 2021.

3.2 Functionality

The CellKb Immune web interface allows users to search or browse cell types and their marker gene sets.

1) Search matching cell types by gene list

CellKb Immune can be used to annotate cells given a ranked list of cluster-specific genes from single-cell experiments. Associated values such as log fold changes and p-values are also accepted. Genes in the user query are compared with every marker gene set in the database and a match score is computed. Other statistics such as the Fisher's exact test, Pearson's correlation coefficient and Jaccard index are also calculated for each cell type match. Ranks of common genes in the user gene list and matching cell types are provided.

The search result shows the matching cell types along with the annotations extracted from their publication (tissue, experimental condition, disease, Pubmed ID, ontology terms), in addition to a heatmap of the match score assigned to each gene for each cell type. Search results are available for download.

2) Search matching cell types by keyword

CellKb Immune can also be used as a reference database to find previously published datasets related to a specific experimental condition, cell type, gene, tissue or disease via the keyword search.

3) Browse cell types

The Browse functionality in CellKb Immune is an index using which users can drill down through categories and find the cell type of interest and their marker gene sets, experimental annotations and publication information.

3.3 Evaluation

To check the database quality and cell type matching performance of the CellKb Immune search method, we used the leave-one-out approach. We compared each of 1,510 marker gene sets with cell ontologies in CellKb Immune with all others in the database. 1,111 (73.6%) of gene sets were correctly matched to another gene set with the exact same ontology or its parent, while for 268 (17.8%) gene sets, the ontology of the first hit was a sibling i.e. a descendant of the same parent ontology (Table 1). Thus, 91.4% of marker gene sets were matched to another cell type with the same ontology, or its parent or sibling (Table S1).

We further evaluated the performance of the CellKb Immune using marker gene lists from Immgen[15] (which is not included in CellKb Immune). Using the most significantly upregulated

genes for each cell type in Immgen, we searched the CellKb Immune database for matching cell types. We then compared the cell ontology of the top 10 hits with that of the ontology assigned to the cell types in Immgen. Of the 276 cell type signatures representing 77 distinct cell types in Immgen, CellKb Immune was able to find the exact matching ontology or its parent as the first hit in 143 instances (51.8%). In another 41 cell types (14.9%), the ontology of the first hit was a sibling i.e. a descendant of the same parent ontology as that in Immgen (Table 1). Thus, a total of 66.7% of cell types in Immgen had their first hit with the same ontology, its parent or sibling (Table S2). This performance is expected to improve as data in CellKb Immune increases.

These results show that 1) the CellKb Immune database includes high quality cell type signatures from single-cell experiments, and 2) the search methodology used by CellKb Immune to find matching cell types is able to recapitulate the original cell types from within its own database, and to some extent, from Immgen.

4. Discussion

CellKb Immune addresses several issues in existing single-cell reference databases. 1) Reanalyzing the raw expression patterns using a common pipeline, as is done by several reference databases, ignores the cluster definitions assigned by authors in the original study. User-defined marker gene sets in literature carry significant knowledge since the cell types in the form of cell clusters are often selected by authors based on biological information. CellKb Immune is able to capture and aggregate this biological information. 2) CellKb Immune provides deep annotations, in the form of extensive cell type information and description, curated and validated marker genes ranked by significance, along with fold change and significance values associated with gene expression. 3) CellKb Immune provides a web-based interface to find matching cell types in published data sets given a user gene list, independent of the experimental platform, analysis methods and insufficient marker gene sets. Thus, users do not need to spend time integrating data and search methods programmatically. 4) Unlike other methods which require the presence of associated expression or fold change values and the same number of genes in all target cell types, the search method used by CellKb Immune enables searching through marker gene sets of differing size, in the absence of expression fold changes and independent of experimental platform and pre-processing methods. Searching of cell types happens by direct comparison of the query gene list with the marker genes of every cell type in CellKb Immune using a fast and reliable method without any type of score aggregation.

Thus, CellKb Immune provides an easy-to-use reference database with a fast and reliable method to find matching cell types and annotate cells in single-cell experiments in a single tool.

Data availability: All data in CellKb Immune is collected from publicly available datasets and is appropriately referenced at https://www.cellkb.com/immune.

Conflict of Interest: Ashwini is CEO at Combinatics Inc., a privately-held company. Ajay and Ashwini are stock-holders in Combinatics Inc.

5. References

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6. Tables

Table 1. Performance evaluation of CellKb Immune

Evaluation database	**Marker gene sets**	**CellKb Immune performance**		
		Correct ontology match	**Parent ontology match**	**Total ontologies matched**
CellKb Immune (leave-one-out)	1,510	1,111 (73.6%)	268 (17.8%)	**1379 (91.4%)**
Immgen (all-against-all)	276	143 (51.8%)	41 (14.9%)	**184 (66.7%)**

7. Figures

Figure 1. CellKb Immune workflow – data collection, harmonization and search interface.

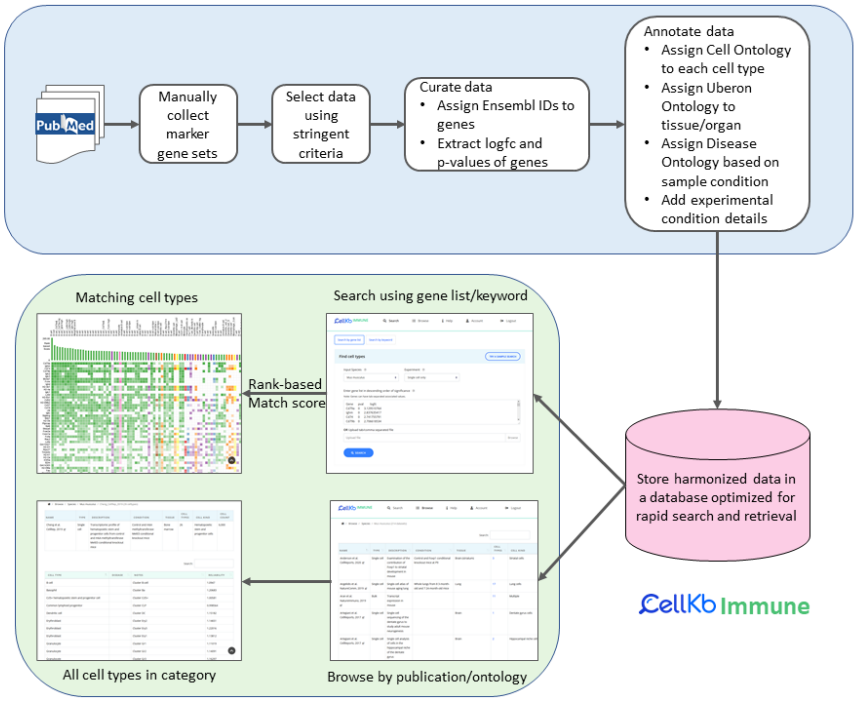
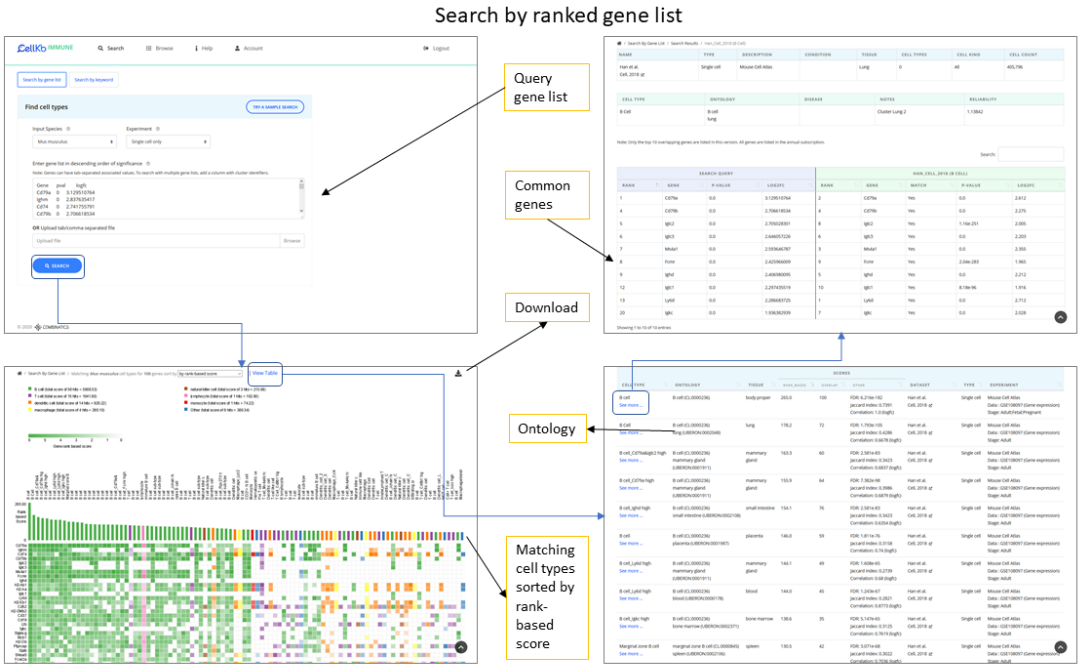
Figure 2. CellKb Immune user interface used to search matching cell types given a gene list.