Originator: Computational Framework Separating Single-Cell RNA-Seq by Genetic and Contextual Information

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Abbreviations:
CCC: Cell-cell communication
DE: Differential expression
GSEA: Gene set enrichment analysis
PBMC: Peripheral blood mononuclear cells
PDAC: Pancreatic ductal adenocarcinoma
QC: Quality control
scRNA-Seq: Single-cell RNA sequencing

Keywords: scRNA-Seq, blood cell, genetic origin, tissue heterogeneity, placenta, cancer

Abstract

Single-cell RNA sequencing data from complex human tissues are prone to blood contamination in sample preparation, and some comprise cells of different genetic makeups, necessitating rigorous preprocessing and cell filtering prior to the downstream functional analysis. Our proposed new computational framework, Originator, deciphers single cells by genetic origin and separates blood cells from tissue-resident cells. It improves the quality of data analysis, exemplified by pancreatic ductal adenocarcinoma and placenta tissues.

Main

Single-cell RNA sequencing (scRNA-Seq) data analysis of complex (*e.g.* placenta) or inherently mixed (*e.g.* tumor) tissues pose significant challenges in computational biology. For example, in tumor studies, precisely separating blood and tissue-resident immune cells in tumor tissues is crucial for understanding the tumor microenvironment [1]. In placenta tissues interfacing with the mother and fetus, isolating the maternal and fetal cells is pivotal to reveal cellular and immunological differences between them [2]. Moreover, many tissues contain blood vessels, and the resulting scRNA-seq data often include blood cell types (e.g., T cells) having highly similar expression profiles with the same cell types in the resident tissues. Therefore, a rigorous preprocessing step is required to enhance the quality of the data of interest, subsequently improving the downstream analysis. Here, we propose a novel computational pipeline called Originator. It deconvolutes barcoded cells into different origins using inferred genotype information from scRNA-Seq data, as well as separating cells in the blood from those in solid tissues, an issue often encountered in scRNA-Seq experimentation.

Fig. 1. Illustration of Originator framework. The input data are the scRNA-Seq experiment on tissue sections. (1) data preprocessing and cell type annotation. (2) separating barcoded cells into different origins by blood vs tissue residents context and optionally by inferred genotype information. (3) using the results in steps (1) and (2) to dissect tissue heterogeneity. (4) the functional downstream analyses with respect to cells' origins.

The proposed Originator framework is illustrated in **Fig 1**. Cells undergo standard scRNA-Seq preprocessing steps, including quality control (QC), normalization, data integration, clustering, and cell-type annotation. Then, Originator utilizes the differences in the genotype information from the scRNA-Seq reads to separate the barcoded cells into different (default N=2) genetic origins, for example, maternal vs. fetal origins. Next, given that many tissues have blood contamination, the subsequent step separates immune cells by blood vs. tissue-resident context using the publicly available whole blood scRNA-Seq data as the reference [3]. We validated the pipeline on artificially mixed data from a PBMCs dataset and a cell mixture dataset containing three breast cancer lines (T47D, BT474, MCF7), monocytes, lymphocytes, and stem cells **(Supplementary Notes)** [4, 5, 6]. As shown by the UMAP plot in **Supplementary Figure 1,** Originator separates the two compartments highly accurately, with AUC of 0.97, F-1 score of 0.97 and Area under the Precision-Recall Curve (AUCPR) of 0.96 (**Supplementary Table 1)**. After dissecting the heterogeneity of the scRNA-seq data, various downstream functional analysis can be performed.

We first applied Originator to a pancreatic ductal adenocarcinoma (PDAC) scRNA-seq dataset [7]**,** to demonstrate its utility in removing cells that originate from blood. Originator successfully separates immune cells in the blood from those in the tumor tissue **(Fig 2a),** and mast and Myeloid-derived suppressor cells (MDSCs) are exclusive in tissues as expected. The immune cell type proportions of the two compartments are shown in **Fig 2b.** The blood has larger

proportions of macrophages and regulatory T-cells, but fewer T-cells. We then performed the differential expression (DE) analysis comparing the common immune cell types between the blood and tumor tissues, and detected significant differences in gene expression in each immune cell type, especially for macrophage and T-cells **(Fig. 2c, Supplementary Table 2-5)**. For example, CCL4 and CCL5 expression is higher in T-cells in the tumor tissue compared to those in the blood (**Supplementary Figure 2a**), consistent with the previous study that these genes are directly associated with T-cell-inflamed phenotype and antigen-presenting cell mediated processes in PDAC [8]. We subsequently performed Gene Set Enrichment Analysis (GSEA) comparing the immune cells' gene expression difference between the blood vs tissue compartments **(Fig. 2d)**. Toll-like receptor (TLR) signaling pathway and NOD-like receptor signaling pathway are more active in tissue-resident T-cells and macrophages compared to those in blood. However, natural killer cell-mediated cytotoxicity signaling pathway is downregulated in NK cells from the tumor compared to the blood compartment, consistent with observed NK cell dysfunction in PDAC via the reduced cytotoxic granule components, granzyme B, and perforin [9]. To demonstrate the impact of blood cell contamination in functional interpretation, we inferred cell-cell communications CCC in tumor tissues before and after removing blood cells **(Fig 2e,f).** While the interactions among the cell types are globally less frequent/noisy in the tumor compartment, those between fibroblast/epithelium and other cell types are strengthened in blood cells, as reflected by the increased node size of these two cell types **(Fig 2e)**. Such a trend of change is most drastic for the MIF signaling pathway (**Fig 2f**), which was previously reported to drive the malignant character of pancreatic cancer [10]. Particularly, MIF signaling between T-cells/regulatory T-cells and other cell types (eg. epithelial cells) is absent after removing blood immune cells, indicating a compromised anti-tumor immune response in PDAC [11].

Some tissues may have different genetic lineages. For example, the placenta has both maternal and fetal tissues. We next applied Originator to the human placenta, and separated the single cells into fetal and maternal origins as well as blood vs. tissue-resident cells. The cells from the fetal origin account for the majority of the cell populations, and the tissue-resident cells significantly over-weigh blood cells, as expected **(Fig 2g)**. The framework correctly and exclusively assigns trophoblast cells, including cytotrophoblasts, syncytiotrophoblasts, and extravillous trophoblasts, to the fetal tissue (**Fig 2g,2h**) [12]. On the contrary, immune cells, vascular endothelial cells, and fibroblasts (type 1 and type 2) appear in both maternal and fetal tissues, also as expected **(Fig 2h)**. We further performed DE analysis on the common cell types between fetal and maternal tissues and discovered drastic differences in gene expression related to the local tissue environment **(Fig 2i, Supplementary Table 6-8).** For example, The top-ranked DE gene EGFL6 between maternal vs. fetal fibroblast cells is highly expressed in fibroblasts subtype 1 in fetal tissues as expected (**Supplementary Figure 3a**) [13]. However, it is much lower in fibroblast subtype 1 or mostly absent in fibroblast subtype 2 in maternal tissues, similar to a previous report [14]. Among the DE genes from macrophage cells (**Supplementary Table 8**), SEPP1 is expressed at much higher levels in Hofbauer cells originating from the fetal tissue compared to the maternal tissue (**Supplementary Figure 3b)**, in accordance with a previous study [15]. We additionally performed GSEA on the DE genes of the common cell types between fetal and maternal cells in the placenta tissue **(Fig 2j)**. The MAPK signaling pathway is enriched in vascular endothelial cells from the fetal tissues, which agrees with its role in growth factor-induced fetoplacental angiogenesis [16]. On the other hand, fibroblast type 2 from the fetal origin is enriched with several other signaling pathways, such as calcium, hippo, and Ras signaling pathways as well as ECM-receptor interaction **(Fig 2j),** which indicates extracellular matrix (ECM) remodeling during trophoblast differentiation [17]. We also compared the CCC among the common cell types between the fetal and maternal tissues **(Fig 2k, 2l)**. Type 1 and type 2 fibroblasts both show different degrees of CCC with some other cell

types when comparing the maternal and fetal tissue contexts. A higher interaction between fibroblast cells (type 1 and type 2) and vascular endothelial cells in fetal tissues reflects the active formation of the villous stroma underneath the syncytiotrophoblast and surrounding fetal capillaries [18]. On the contrary, higher interactions between type 2 fibroblast and macrophages in maternal tissue may assist trophoblast invasion through growth factors and cytokines [19]. Thus teasing apart the cell types by their genetic and local tissue context helps to refine the molecular analysis in placenta scRNA-seq data.

In conclusion, we demonstrate that Originator can systematically decipher scRNA-Seq data by genetic origin and blood/tissue contexts in heterogeneous tissues, exemplified by PDAC and placenta. It can be used as an effective tool to remove the undesirable blood cells in scRNA-Seq data. It can also provide improved cell type annotations and other downstream functional analyses, based on the genetic background.

Fig. 2. Applications of Originator to pancreatic ductal adenocarcinoma (PDAC) and placenta scRNA-seq data. a, UMAP plot of cells in PDAC tissues (top-left) separated into blood and tissue-resident immune cells (bottom-left). UMAP plot of cells in PDAC tissues after blood immune cell removal is shown on the top-right. **b,** The cell type proportion barplot shows the immune cell types in blood and PDAC tumor tissue, as well as all cell types in the tumor tissue before and after blood immune cell removal. **c,** Venn diagram of the significant DE genes identified when comparing immune cells in blood and tumor tissue. **d,** GSEA results comparing blood and tissue-resident immune cell types. **e,** Overall CCC in the tumor tissues before and after blood immune cell removal. **f,** CCC of MIF signaling pathway in tumor tissues before and after blood immune cell removal. **g,** UMAP plot of cells in placenta tissues deciphered into fetal vs. maternal origins and blood vs. placenta-resident cells. **h,** Cell type proportions in the different compartments related to the placenta. **i,** Upset plot of significant DE genes identified in common cell types between fetal and maternal placenta tissues. T-cell is excluded due to the near 0

counts in the fetal tissue. **j,** GSEA results comparing fetal and maternal tissues among common cell types. **k-l,** CCC of common cell types in fetal (**k**) and maternal (**l**) tissues.

Methods

scRNA-Seq case study data sets

Two datasets are used in this report. One is a PDAC scRNA-Seq dataset, in which two tumor tissues are used in this study (GSE212966) [7]. The other is the scRNA-Seq data from placenta tissues,comprising eight placenta samples from the previous study (EGA; https://www.ebi.ac.uk/ega/) hosted by the European Bioinformatics Institute (EBI; accession no. EGAS00001002449) [20]. Cell types in the placenta and PDAC tissues are annotated using the cell-type-specific marker genes (**Supplementary Table 9-10**).

Description of Originator for scRNA-Seq data analysis

Originator is a multi-module framework that can be used to preprocess scRNA-Seq data from heterogeneous tissues. It consists of one mandatory step based on the tissue vs blood compartments and also an optional step based on the genetic origins. Originator takes the gene expression matrix processed by Cell Ranger (version 7.1.0) of 10x genomics as the input [21]. For genetic background based deciphering, it also uses the BAM file. It processes and outputs the R data serialization file (RDS). This output file contains gene expression and related information, including the cell type, blood and tissue immune cell annotation, and annotation by genetic information if this function is needed.

First, Originator separates immune cells in blood vs. those in the tissue using the blood immune cell scRNA-Seq reference constructed from the publicly available scRNA-seq data from the whole blood cells [3]. Annotated immune cells of interest (monocytes, macrophages, T-cells, regulatory T-cells, plasma cells, NK cells, and B-cells) from the datasets of this study are

aligned to the whole blood scRNA-Seq reference data using package Seurat (4.3.0) [22]. For each immune cell type, the top 10 embeddings from the UMAP analysis of each scRNA-Seq sample are used to compute a pairwise Euclidean distance matrix between each query immune cell and the reference whole blood. To separate each query of immune cells into blood or tissue-resident cells, k-means clustering (k = 2) is applied using Euclidean distances relative to the whole blood immune cell reference. The cluster more similar to the whole blood immune cell references is annotated as the blood immune cells, and the other cluster is determined as the tissue-resident immune cells.

If the tissue contains the mixture of cells of different genetic background, such as maternal and fetal origins, Originator provides an optional step to decipher the barcoded cells by the genetic origin relies on the overlapping genetic variants extracted from the scRNA-Seq data. Genetic variants are extracted by *bcftools* (version 1.17) [23]. Variants informative of genetic origins are determined by excluding single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) greater than x percentages (default x=10), using the 1000 Genomes Phase 3 reference panel (release date: 2013/05/02) [24]. The *freemuxlet* package is used to separate the single cells with the parameter '--nsample N' (default N=2), as implemented in popscle (https://github.com/statgen/popscle) [25]. N denotes the number of genetic origins of the single cells (e.g., N = 2 for the maternal and fetal origins in a placenta scRNA-Seq data). Mosaic doublets (a single barcoded cell with a 50% genetic mixture from two individuals) are also identified in this step as part of the quality control. As trophoblast cells in the placenta are of fetal origin, the cluster that contains trophoblast cells is identified with the fetal origin, while the other cluster is marked as the maternal origin.

Simulation

The artificially mixed tissue-blood-resident data was generated to assess the ability of Originator to separate blood cells and tissue immune residents. We included only mixture 1

(GSM6807287) in the simulated data to reduce the computational cost during the origins and tissue-blood deconvolution process (**Supplementary Notes**) [6].

Downstream analysis

DE analysis was performed using *FindMarkers()* in the package Seurat (4.3.0) [22]. GSEA is done using *gseKEGG()* in the package ClusterProfiler (4.8.2) with the gene set information from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [26, 27]. CCC inference was performed using the package CellChat [28].

Code availability

Originator is freely available to use at URL: https://github.com/lanagarmire/Originator

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Author Contributions

L.X.G. and Q.H. conceived this project and supervised the study. T.U. modified the pipeline, carried the analysis, and wrote the manuscript. Q.H. wrote the Originator pipeline. M.Z., Y.Y., and Y.D. assisted the analysis and tested the package.

Competing Interests

LXG is a member of the Scientific Advisory Boards of Simulations Plus.

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Fig. 1. Illustration of Originator framework.

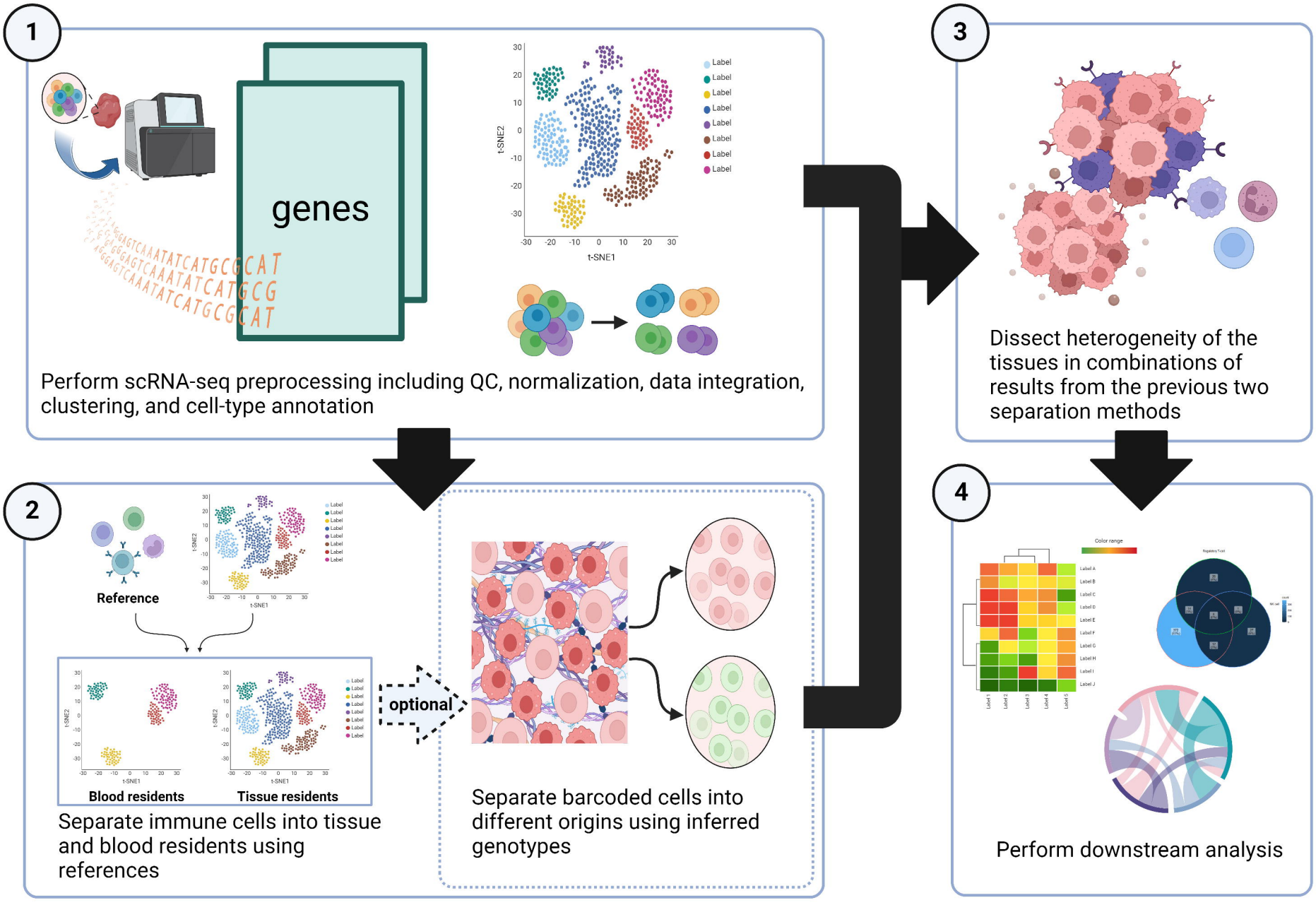
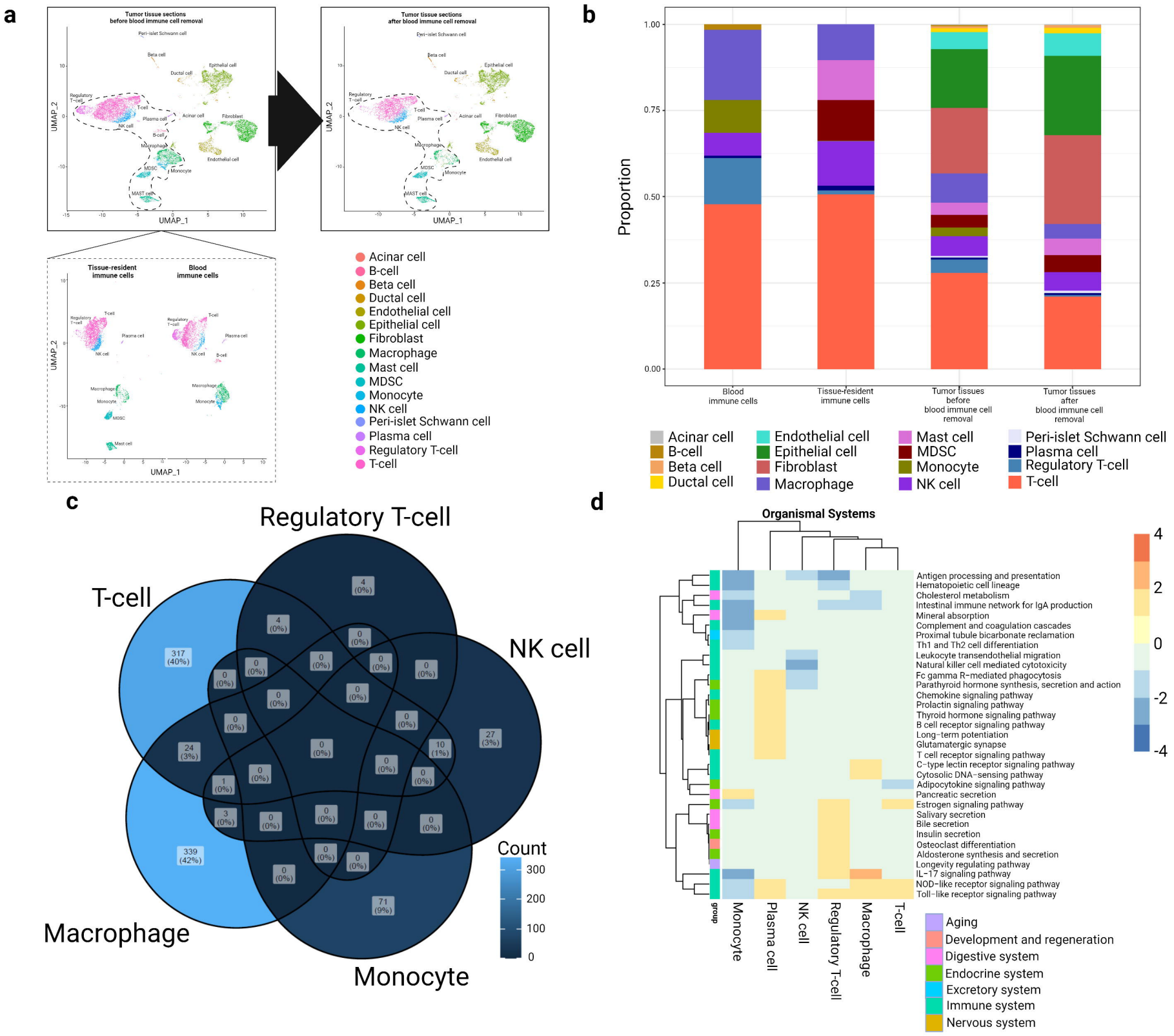
Fig. 2. Applications of Originator to pancreatic ductal adenocarcinoma (PDAC) and placenta scRNA-seq data.