SPRUCE - Single-cell Pairwise Relationships Untangled by Composite ETM

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Introduction

The advancement in single-cell RNA-sequencing (scRNA-seq) has emerged as a new frontier in genomics. Quantification of multimoldal omics at a single-cell resolution has made it possible to gain insights into different aspects of cancer biology (Teichmann and Efremova [2020]). One of the fundamental questions is - how cells interact with each other in confined heterogeneous environment such as tumour microenvironment (TME)? Studies in the past have shown that the cell-cell communication(CCC) among cell populations in the TME is crucial in cancer growth and metastatic processes (Tan and Naylor [2022]). Understanding the intricacies of communication among tumour and their interacting partner cells could aid in identifying potential therapeutic avenue in cancer.

A major challenge in understanding the dynamics of cell-cell interactions in TME is devising a systematic approach to isolate and capture interaction signal from each interacting cell-pair. A conventional approach to studying CCC involves clustering features in low-dimensional space and inferring interactions between clusters of know cell type (Almet et al. [2021], Jin et al. [2021], Efremova et al. [2020]). While these methods have uncovered numerous signalling mechanisms that govern cellular differentiation and pathogenesis, they assume each cluster, annotated using a limited number of marker genes, represents a cell type and all the cells within a cluster interact in the same manner. These methods do not account for intracluster cellular heterogeneity. Cells

within a cell type may exist in multiple subtype/state and manifest heterogeneous interaction patterns based on the type and state of interacting partner cell, which is critical in understanding cancer progression (Zhang et al. [2021a], Tan and Naylor [2022]). Additionally, interaction among cells in different contexts, such as disease states, are studied separately, which loses context-specific variability information and are repetitive and computationally expensive.

Recent studies have addressed these challenges and developed methods to capture the diversity of cell interactions within the same cluster. Tensor-cell2cell (Armingol et al. [2022]) uses tensor based dimensionality reduction techniques to infer context driven CCC pattern. scTensor (Tsuyuzaki et al. [2019]) also uses a tensor decomposition algorithm to infer many-to-many cell-pair relationships as a hypergraph. These methods rely on a priori knowledge of cell type and aggregating cells to calculate communication scores based on the mean expression of ligand receptor (LR) genes. SoptSC (Wang et al. [2019]) calculates signalling probability between two cells based on pathway-specific LR and target genes and addresses heterogeneity of cells within the same cluster. However, the method requires a user-defined comprehensive list of pathway genes and does not scale to cohort-level studies.

Here,we introduce a scalable, integrative, and biologically interpretable computational approach SPRUCE, Single-cell Pairwise Relationship Untangled by Composite ETM, that can untangle CCC at cell-pair resolution. SPRUCE is based on an embedded topic model (ETM) which is a generative deep learning method that uses variational autoencoder architecture to represent data in low-dimension topics with an interpretable gene to interaction pattern relationship. It has been successfully implemented in natural language processing to extract meaningful topics representing large-scale documents (Dieng et al. [2020]). In a recent study, scETM showed that ETM-based techniques efficiently capture the essential biological signals from sparse and heterogeneous single-cell data (Zhao et al. [2021]). The key contribution of our approach is the unbiased identification of interpretable cell subtype/state across multiple datasets and characterization of LR genes driven pattern of cell-cell interactions. The SPRUCE learns cell-cell interaction network from edge's perspective where millions of cell-pair gene expression signal is used to learn network parameters and biologically interpretable embeddings of interaction pattern.

Results

Overview of the SPRUCE approach for characterizing interaction patterns between cells

The systematic analysis of cellular composition and cell-cell communication in the TME is crucial in understanding the complex biological mechanisms behind cancer progression (Binnewies et al. [2018]). In developing our model, we hypothesize that two layers of information are critical in deciphering the multifaceted nature of CCC in the TME. First, we are interested to disintegrate cellular heterogeneity by identifying a subtype/state of each cell - unbiased transcriptional signature. Since the clustering of cells based on low-dimensional representation of differentially expressed genes (DEGs) loses the information from majority of unselected genes, we sought for de novo clustering approach in which we capture information from all available transcriptomics without the need of dimension reduction and DEGs. Second, we assume that cell-cell interaction occurs between a cell pair with the same or different transcriptional signature. Specifically, we predicted that comprehensive pairing of a source cell with target cells representing all transcriptional signatures is essential in inferring the global interaction patterns in the TME at a cell-pair resolution. Accordingly, we developed SPRUCE, a probabilistic deep learning method that groups heterogeneous cell populations into distinct cell subtypes/states and identifies unique interaction patterns represented in the TME. Briefly, the model is composed of two steps - the first step represents each cell in cell topic space, and the next step generates cell pairs based on cell topic assignment and represents each pair in interaction topic space. As the final output, the model extracts pair-wise LR-driven interaction patterns in an unsupervised manner. The cell and interaction topics are informative and biologically interpretable low-dimensional representations of cell topics and their dynamic communication pattern.

The model was trained using combined breast cancer data sets with ~155K cells from multiple large-scale studies. First, the model represented all the cells into 50 cell topics, with various topics represented as unique cell topics/states of different cell types, including cancer cells. To represent all the components of TME and capture the extensive crosstalk among tumours, resident cells, and recruited immune cells, we constructed a list of ~25 million neighbour cell pairs where neighbours

of each cell represented all cell topics. We then transformed LR gene expression data from each cell pair and represented it in lower dimension of 25 interaction topics. Using SPRUCE, we identified seven different interaction topics with unique set of LR gene loadings representing tumour-immune, tumour-stromal, and tumour-tumour interactions. The model extracted interaction patterns to represent dynamic cell-cell communication among different cell types present in various cell subtypes in the TME.

Multinomial probabilistic topic modelling identified 50 cell topics across 11 known cell types

We implemented a Bayesian deep learning approach to estimate embedded topic models across 155,913 cells with 50 latent dimensions. We found each cell topic corresponds to a group of average 3118 cells (with standard deviation $\pm$ 5987) (Figure 1A). Among 50 topics, 32 of them contained more than 100 cells. The highest number of cells (24% of the dataset) were assigned to topic 37 in which 96% of cells were previously identified immune cells (T and B cells). 98% of cancer cells from the dataset were assigned to 13 cell topics. The cancer cell proportion in 9 of 13 topics was greater than 95%. The latent cell topics with cell type annotated were visualized with UMAP, which shows distinct clusters for each topic where the majority of cells belong to one of the major types of cells in the dataset (Figure 1B). This was further confirmed using cell type lineage canonical markers (Figure 1D). The estimated cell topic proportions show that the resident cell types have similar topic proportions. However, cancer cells have a different mixture of topic proportions which shows that the model identified many distinct topics of cancer cells (Figure 1E). We also tried different number of cell topics from 10, 25, 50 and decided to use the 50-topic model because major cell types, especially cancer cells, showed well separated distinct clusters.

Common signatures of 25 million cell-cell pairs

We constructed LR gene expression data from 155,913 cells to construct a set of 24,790,167 cell pairs and estimated embedded interaction topic models with 25 latent dimensions. Among 25 interaction topics representing ~25 million cell pairs, seven topics (2,4,7,10,18,22, and 24) represented 55% of the total cell pair interactions, with each topic containing >3% cell pairs (Figure 2C). The other 18 interaction topics, each with ~2% of the total cell pair interactions embedded baseline interaction

signal. The most represented interaction topic was topic 22, consisting 12% of the total cell pair interactions.

The model estimated the LR gene loadings in each interaction topic that described the relative contribution of each gene. These loadings can be ranked to identify biologically interpretable topic-specific top genes in each interaction topic (Figure 2D). Topics, 22 and 24, captured immune-related interactions. Topic 22 was labelled as lymphoid associated topic because top receptors included subunit of T-Cell Receptor Complex CD3D and killer cell lectin like receptors KLRC1, KLRC2, and KLRD1. The top ligands in this topic are HLA-E, CLEC2B, and CLEC2DC, which are essential known modulators in cytotoxic T cells (Dufva et al. [2020]). Similarly, topic 24 was labelled as myeloid associated topic as top genes in this topic showed enrichment of LR genes expressed by myeloid progenitors, for example - receptors such as CD68, TREM2, and CR1, and ligands such as CCL23, CCL18, CCL13, and C1QA (Hussain et al. [2021]).

Topics 10 and 7 represented many oncogenes mutated in cancer- topic 10 was cancer-growth associated, and genes that play a role in cancer cell survival and growth are enriched in this topic. The top receptors in this topic are growth factor receptors such as ERBB2, cell proliferation and growth signalling receptor FZD10, and immune inhibiting signalling receptor ADORA2A (Miller et al. [2015], Cekic and Linden [2014]). Similarly, topic 7 was cancer-metastasis associated topic and genes such as NTRK3, known to increase the metastatic potential of cancer cells, GRPR, which promotes EMT, and UNC5A, a known regulator of cancer plasticity, are enriched in this topic (Zhang et al. [2021b], Elshafae et al. [2016], Padua et al. [2018]).

Further, topic 18 was stroma-associated and represented genes that play an integral role in regulating the extracellular matrix (ECM) of the tumour immune microenvironment. These genes are highly expressed in cancer-associated fibroblast (CAF) and perivascular-like (PVL) cells. The top ligands in this topic are COL1A1, COL1A2, COL3A1, and MMP13, and the top receptors are ITGA11 and SCARA5 (Primac et al. [2019], Bansal et al. [2017]). Similarly, endothelial-associated topic 2 is enriched with genes highly expressed in endothelial cells. Here, endothelial associated ligands such as CD34, ANGPT2, and NID2 and receptors such as APLNR and ESAM are enriched (Wu et al. [2017]). Likewise, topic 4 was TME-regulation associated topic enriched in genes that promote a conducive environment for cancer growth. The top genes in this topic are KISS1R/KISS1,

which play complex role in both restricting and promoting cancer cell survival, IL20RB, which promotes immunosuppressive microenvironment, and MMP24, which negatively regulates the aggressiveness of cancer cells (Cvetković et al. [2013]).

The top LR genes in the major interaction topics show enrichment of different cell type specific functional interactions. To confirm that each interaction topic captured cell type specific CCC, we took a closer look at the distribution of cell types of neighbour cells in each interaction topic for all the cells in the dataset. We found that the functional role of enriched top LR genes in each interaction topic matched with the dominant cell type of neighbour cells in that topic (Figure 2E). For example, in the cancer-growth associated, on average, 68% of neighbour cells for all cell types were cancer cells. Similarly, 49% of neighbour cells in stroma-associated topic were CAF/PVL cells, and myeloid and T cells comprised of 49% and 38% of neighbour cells in myeloid-associated and lymphoid-associated topics, respectively. For endothelial-associated topic, dominant neighbour cell type was endothelial cells with 18%. In contrast, for TME-regulation associated topic, both epithelial and plasms cells were dominant cells consisting of 20% and 22%, respectively.

In addition, the cell type specific enrichment of interaction topic was further corroborated by the distribution of interaction topics in each cell type. Cancer cells along with epithelial, plasma, and B cells showed heterogeneous interaction patterns compared to myeloid, T, endothelial, CAF, and PVL cell types (Figure 2F). For cancer cells, the majority of interactions belonged to cancer-growth associated and cancer-metastasis associated topics where many of the top genes were oncogenes. Here, 55% of the total interactions was cancer-growth associated, and 17% belonged to cancer-metastasis associated topic, while the other five remaining topics consisted of 3-8% of interactions. Among the non-cancer cell types, the dominant interaction topic for myeloid cells was myeloid-associated interaction topic and for T cells it was lymphoid-associated interaction topic. Here, 88% of myeloid cell and 65% of T cell interactions were found to be in respective interaction topic. Similarly, 83% of cell interactions with endothelial cells belonged to endothelial-associated topic, and 77% and 53% of interactions with CAF and PVL cells, respectively were stroma-associated topic. In contrast, plasma, B, and epithelial cells showed a higher mixture non-immune associated interaction topics.

Heterogeneity of breast cancer cells defined by topic-specific interaction patterns

Next, we investigated the heterogeneity of breast cancer cells based on the unbiased transcriptomic signature captured by cell topic model and all possible functional interactions of cells in the microenvironment uncovered by the interaction topic model. The interaction patterns of 25,835 cancer cells manifested all the patterns of interactions (Figure 3B). The cell topic model identified 13 cell topics for cancer cells that show a distinct pattern of interactions with their neighbouring cells (Figure 3C). The cancer-growth associated topic was the most dominant (>57%) among 7 of 13 cell topics. For instance, 75%,70%, and 68% of interactions for cancer cells in cell topics 24, 48, and 2 belonged to cancer-growth associated interaction topic. There were two cell topics in which major interactions were non-cancer like topics- 66% of interactions in cell topic 9 consisted of stroma-associated topic and 60% of interactions in cell topic belonged to endothelial-associated interaction topic. When we compare other cell types - T, myeloid, endothelial, CAF, and PVL cell types did not deviate from cell type specific interaction pattern, while cell topics from plasma, B, epithelial showed significant variability of interaction patterns among cell topics.

Breast cancer cells are classified into subtypes based on the genomics and pathology of the disease that show correlation with clinical outcomes (Horr and Buechler [2021]). All three different subtypes of cancer cells show diverse interaction patterns where TNBC cancer cells were more heterogeneous compared to HER2+ and ER+ subtypes (Figure 3D). Here, more than 85% of interactions of HER2+ subtype consisted of cancer-associated topics - 82% for cancer-growth and 5% for cancer-metastasis associated topics. Similarly, for ER+ subtype, more than 80% of interactions were cancer-associated topics - 61% for cancer-growth and 20% for cancer-metastasis associated topics. In contrast, TNBC subtype cells were more diverse in interactions, with 42% for cancer-growth associated, 15% for cancer-metastasis associated, 15% for stroma-associated, and 10% for myeloid-associated topics. Additionally, our approach identified a specific group of cells (cell topic) within these cancer subtype that show topic-specific interaction patterns. At the cell topic level, TNBC cell topics show higher heterogeneity in interaction patterns compared to ER+ and HER2+ subtypes. The disribution of interaction pattern among cell topics is correlated with the expression pattern of LR genes enriched in each interaction topic. For example, TNBC cancer cells in cell topic 9 show higher expression of LR genes enriched in myeloid-associated interaction topic, while cancer-growth

associated LR genes are dominant among TNBC cancer cells in cell topic 24 (Figure 3E).

Different interaction topics induce subtype-specific gene-gene networks

We generated a topic-specific gene correlation network with significantly expressed LR genes to investigate intercellular communication between cancer cells and their neighbours in different interaction topics. In lymphoid-associated topic, major components of T cell receptor (TCR) complex (CD3D,CD3G, CD2, and CD247) and genes involved in regulating TCR signaling pathway PTPRC, CD45, and CD53 are abundantly enriched, suggesting the regulatory interactions between cancer and T cells (Shah et al. [2021]). Other genes enriched in this topic are involved in crosstalk between T cells and cancer cells and promote cancer growth and proliferation in the tumour microenvironment. For example, the chemokine receptors CXCR3 and CXCR4 are known to mediate metastasis of breast cancer cells and killer-cell lectin like receptors KLRC1, KLRD1, and KLRF1 are known to restrict T-cell's antitumour immunity (Kuo et al. [2018], Hu et al. [2021]). Similarly, immune-modulatory receptors primarily expressed in myeloid lineage cells TREM2, CSF1R, CSF2R, LILR, and IL3R and signalling pathways LTBR and TYROBP required for the activation of myeloid cells are associated with myeloid-associated interaction topic. This topic captures the interactions between cancer cells and myeloid cell progenitors such as tumour-associated macrophages (TAM) in the tumour microenvironment, suppressing T cells and facilitating tumour growth (Molgora et al. [2020]).

The gene interactions in two cancer associated topics showed that genes enriched in cancer-growth associated topic captured the interactions between cancer cells and other cell types that promote its growth, while genes in cancer-metastasis associated topic were active in cancer cell transformation and metastasis. The dominant gene network in cancer-growth associated topic consisted of highly upregulated genes that induces signaling cascades involved in oncogenesis such as PTPRF, FGFR1, ERBB2, and TNFRSF1A (Butti et al. [2018]). Also, genes known to play an essential role in cellular developmental processes and hijacked by cancer cells, such as LAMP1, ITGB1, RPSA, CANX, ATP6AP2, and MCFD2 are enriched in this topic (Going et al. [2018]). Similarly, gene networks in cancer-metastasis topic consisted of a group of structural genes CLDN4, LSR, and DSG2 involved in cell transformation and migration and signalling pathways GPR37, CD151, and

CD63 that are active in proliferation and migration, including epithelial-mesenchymal transition (EMT) (Shang et al. [2012], Wang et al. [2021]).

The stroma-associated topic represented gene networks that capture the interaction of cancer cells with surrounding cells that promote its vascularization. It consisted of NOTCH3, AVPR1A, MYLK and integrin-mediated ITGA1, ITGA5, ITGA7, and ITGB1 signalling pathways that play vital roles in tumour cell adhesion and progression (Price et al. [2020]). The genes MCAM, ENPEP, EDNRA, and DCBLD2 that promote blood vessel formation and enhance tumorigenesis are enriched in this topic (Wragg et al. [2016]). Similarly, genes enriched in endothelial-associated topic captures interactions of cancer cells in developing tumour vascular networks, especially in conjunction with endothelial cells. PECAM1, CALCR, ADGRL4, and CD93 genes that are predominantly expressed in endothelial cells and regulate angiogenesis in tumour cells are present in this topic (Sheldon et al. [2021]). Additionally, TME-regulation associated topic primarily consisted of genes mixture of enthothelial-associated and stroma-associated topics with enrichment of distinct genes known to control tumour growth and promote stemness of cancer cells in microenvironment such as KCNN4, IL6ST, and CD1B (Fan et al. [2022]).

Discussion

The cancer cells are found to express top LR genes in each interaction topic identified by the model. For immune-related interaction topics and stroma-associated topics, cancer cells show higher expression of ligands than receptors compared to their neighbour cells. But for other interaction topics, especially in cancer-growth associated and cancer-metastasis associated topics, cancer cells and their neighbour cells show similar expression patterns of top LR genes.

References

Axel A Almet, Zixuan Cang, Suoqin Jin, and Qing Nie. The landscape of cell–cell communication through single-cell transcriptomics. *Current opinion in systems biology*, 26:12–23, 2021.

Erick Armingol, Hratch M Baghdassarian, Cameron Martino, Araceli Perez-Lopez, Caitlin Aamodt,

Rob Knight, and Nathan E Lewis. Context-aware deconvolution of cell–cell communication with tensor-cell2cell. *Nature communications*, 13(1):1–15, 2022.

Ruchi Bansal, Shigeki Nakagawa, Saleh Yazdani, Joop Van Baarlen, Anu Venkatesh, Anna P Koh, Won-Min Song, Nicolas Goossens, Hideo Watanabe, Mary B Beasley, et al. Integrin alpha 11 in the regulation of the myofibroblast phenotype: implications for fibrotic diseases. *Experimental & molecular medicine*, 49(11):e396–e396, 2017.

Mikhail Binnewies, Edward W Roberts, Kelly Kersten, Vincent Chan, Douglas F Fearon, Miriam Merad, Lisa M Coussens, Dmitry I Gabrilovich, Suzanne Ostrand-Rosenberg, Catherine C Hedrick, et al. Understanding the tumor immune microenvironment (time) for effective therapy. *Nature medicine*, 24(5):541–550, 2018.

Ramesh Butti, Sumit Das, Vinoth Prasanna Gunasekaran, Amit Singh Yadav, Dhiraj Kumar, and Gopal C Kundu. Receptor tyrosine kinases (rtks) in breast cancer: signaling, therapeutic implications and challenges. *Molecular cancer*, 17(1):1–18, 2018.

Caglar Cekic and Joel Linden. Adenosine a2a receptors intrinsically regulate cd8+ t cells in the tumor microenvironmentadenosine maintains cd8+ t cells in solid tumors. *Cancer research*, 74 (24):7239–7249, 2014.

Donna Cvetković, Andy V Babwah, and Moshmi Bhattacharya. Kisspeptin/kiss1r system in breast cancer. *Journal of Cancer*, 4(8):653, 2013.

Adji B Dieng, Francisco JR Ruiz, and David M Blei. Topic modeling in embedding spaces. *Transactions of the Association for Computational Linguistics*, 8:439–453, 2020.

Olli Dufva, Petri Pölönen, Oscar Brück, Mikko AI Keränen, Jay Klievink, Juha Mehtonen, Jani Huuhtanen, Ashwini Kumar, Disha Malani, Sanna Siitonen, et al. Immunogenomic landscape of hematological malignancies. *Cancer Cell*, 38(3):380–399, 2020.

Mirjana Efremova, Miquel Vento-Tormo, Sarah A Teichmann, and Roser Vento-Tormo. Cellphonedb: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. *Nature protocols*, 15(4):1484–1506, 2020.

Said M Elshafae, Bardes B Hassan, Wachiraphan Supsavhad, Wessel P Dirksen, Rachael Y

Camiener, Haiming Ding, Michael F Tweedle, and Thomas J Rosol. Gastrin-releasing peptide receptor (grpr) promotes emt, growth, and invasion in canine prostate cancer. *The Prostate*, 76 (9):796–809, 2016.

Jing Fan, Ruofei Tian, Xiangmin Yang, Hao Wang, Ying Shi, Xinyu Fan, Jiajia Zhang, Yatong Chen, Kun Zhang, Zhinan Chen, et al. Kcnn4 promotes the stemness potentials of liver cancer stem cells by enhancing glucose metabolism. *International journal of molecular sciences*, 23(13): 6958, 2022.

Catherine C Going, Dhanir Tailor, Vineet Kumar, Alisha M Birk, Mallesh Pandrala, Meghan A Rice, Tanya Stoyanova, Sanjay Malhotra, and Sharon J Pitteri. Quantitative proteomic profiling reveals key pathways in the anticancer action of methoxychalcone derivatives in triple negative breast cancer. *Journal of proteome research*, 17(10):3574–3585, 2018.

Christina Horr and Steven A Buechler. Breast cancer consensus subtypes: A system for subtyping breast cancer tumors based on gene expression. *NPJ breast cancer*, 7(1):1–13, 2021.

Ziming Hu, Xiuxiu Xu, and Haiming Wei. The adverse impact of tumor microenvironment on nk-cell. *Frontiers in Immunology*, 12:633361, 2021.

Khiyam Hussain, Mark S Cragg, and Stephen A Beers. Remodeling the tumor myeloid landscape to enhance antitumor antibody immunotherapies. *Cancers*, 13(19):4904, 2021.

Suoqin Jin, Christian F Guerrero-Juarez, Lihua Zhang, Ivan Chang, Raul Ramos, Chen-Hsiang Kuan, Peggy Myung, Maksim V Plikus, and Qing Nie. Inference and analysis of cell-cell communication using cellchat. *Nature communications*, 12(1):1–20, 2021.

Paula T Kuo, Zhen Zeng, Nazhifah Salim, Stephen Mattarollo, James W Wells, and Graham R Leggatt. The role of cxcr3 and its chemokine ligands in skin disease and cancer. *Frontiers in Medicine*, 5:271, 2018.

Martin L Miller, Ed Reznik, Nicholas P Gauthier, Bülent Arman Aksoy, Anil Korkut, Jianjiong Gao, Giovanni Ciriello, Nikolaus Schultz, and Chris Sander. Pan-cancer analysis of mutation hotspots in protein domains. *Cell systems*, 1(3):197–209, 2015.

Martina Molgora, Ekaterina Esaulova, William Vermi, Jinchao Hou, Yun Chen, Jingqin Luo, Si-

mone Brioschi, Mattia Bugatti, Andrea Salvatore Omodei, Biancamaria Ricci, et al. Trem2 modulation remodels the tumor myeloid landscape enhancing anti-pd-1 immunotherapy. *Cell*, 182(4):886–900, 2020.

Maria B Padua, Poornima Bhat-Nakshatri, Manjushree Anjanappa, Mayuri S Prasad, Yangyang Hao, Xi Rao, Sheng Liu, Jun Wan, Yunlong Liu, Kyle McElyea, et al. Dependence receptor unc5a restricts luminal to basal breast cancer plasticity and metastasis. *Breast Cancer Research*, 20(1):1–18, 2018.

Jessica C Price, Elham Azizi, LA Naiche, Jenny G Parvani, Priyanka Shukla, Seoyeon Kim, Jill K Slack-Davis, Dana Pe'er, and Jan K Kitajewski. Notch3 signaling promotes tumor cell adhesion and progression in a murine epithelial ovarian cancer model. *PloS one*, 15(6):e0233962, 2020.

Irina Primac, Erik Maquoi, Silvia Blacher, Ritva Heljasvaara, Jan Van Deun, Hilde YH Smeland, Annalisa Canale, Thomas Louis, Linda Stuhr, Nor Eddine Sounni, et al. Stromal integrin α11 regulates pdgfrβ signaling and promotes breast cancer progression. *The Journal of clinical investigation*, 129(11):4609–4628, 2019.

Kinjal Shah, Amr Al-Haidari, Jianmin Sun, and Julhash U Kazi. T cell receptor (tcr) signaling in health and disease. *Signal transduction and targeted therapy*, 6(1):1–26, 2021.

Xiying Shang, Xinjian Lin, Edwin Alvarez, Gerald Manorek, and Stephen B Howell. Tight junction proteins claudin-3 and claudin-4 control tumor growth and metastases. *Neoplasia*, 14(10):974–IN22, 2012.

Helen Sheldon, Esther Bridges, Ildefonso Silva, Massimo Masiero, David M Favara, Dian Wang, Russell Leek, Cameron Snell, Ioannis Roxanis, Mira Kreuzer, et al. Adgrl4/eltd1 expression in breast cancer cells induces vascular normalization and immune suppressioneltd1 is angiogenic and immunosuppressive in breast cancer. *Molecular Cancer Research*, 19(11):1957–1969, 2021.

Keely Tan and Matthew J Naylor. Tumour microenvironment-immune cell interactions influencing breast cancer heterogeneity and disease progression. *Frontiers in Oncology*, 12, 2022.

Sarah Teichmann and Mirjana Efremova. Method of the year 2019: single-cell multimodal omics. *Nat. Methods*, 17(1):2020, 2020.

Koki Tsuyuzaki, Manabu Ishii, and Itoshi Nikaido. Uncovering hypergraphs of cell-cell interaction from single cell rna-sequencing data. *BioRxiv*, page 566182, 2019.

Jian Wang, Min Xu, Dan-Dan Li, Wujikenayi Abudukelimu, and Xiu-Hong Zhou. Gpr37 promotes the malignancy of lung adenocarcinoma via tgf-β/smad pathway. *Open Medicine*, 16(1):024–032, 2021.

Shuxiong Wang, Matthew Karikomi, Adam L MacLean, and Qing Nie. Cell lineage and communication network inference via optimization for single-cell transcriptomics. *Nucleic acids research*, 47(11):e66–e66, 2019.

Joseph W Wragg, Jonathan P Finnity, Jane A Anderson, Henry JM Ferguson, Emilio Porfiri, Rupesh I Bhatt, Paul G Murray, Victoria L Heath, and Roy Bicknell. Mcam and lama4 are highly enriched in tumor blood vessels of renal cell carcinoma and predict patient outcome. *Cancer research*, 76(8):2314–2326, 2016.

Xinqi Wu, Anita Giobbie-Hurder, Xiaoyun Liao, Courtney Connelly, Erin M Connolly, Jingjing Li, Michael P Manos, Donald Lawrence, David McDermott, Mariano Severgnini, et al. Angiopoietin-2 as a biomarker and target for immune checkpoint therapy. *Cancer immunology research*, 5(1): 17–28, 2017.

Yang Zhang, Tianyuan Liu, Xuesong Hu, Mei Wang, Jing Wang, Bohao Zou, Puwen Tan, Tianyu Cui, Yiying Dou, Lin Ning, et al. Cellcall: integrating paired ligand–receptor and transcription factor activities for cell–cell communication. *Nucleic Acids Research*, 49(15):8520–8534, 2021a.

Zhao Zhang, Yongbo Yu, Pengfei Zhang, Guofeng Ma, Mingxin Zhang, Ye Liang, Wei Jiao, and Haitao Niu. Identification of ntrk3 as a potential prognostic biomarker associated with tumor mutation burden and immune infiltration in bladder cancer. *BMC cancer*, 21(1):1–13, 2021b.

Yifan Zhao, Huiyu Cai, Zuobai Zhang, Jian Tang, and Yue Li. Learning interpretable cellular and gene signature embeddings from single-cell transcriptomic data. *Nature communications*, 12(1): 1–15, 2021.