

Studying immune to non-immune cell cross-talk using single-cell technologies

R. Elmentaite¹, S. A. Teichmann^{1,2,3} and E. Madisson^{1,3}

Abstract

Single-cell RNA-sequencing has uncovered immune heterogeneity, including novel cell types, states and lineages that have expanded our understanding of the immune system as a whole. More recently, studies involving both immune and non-immune cells have demonstrated the importance of immune microenvironment in development, homeostasis and disease. This review focuses on the single-cell studies mapping cell–cell interactions for variety of tissues in development, health and disease. In addition, we address the need to generate a comprehensive interaction map to answer fundamental questions in immunology as well as experimental and computational strategies required for this purpose.

Addresses

¹ Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, CB10 1SA, United Kingdom

² Theory of Condensed Matter, Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge, CB3 0HE, United Kingdom

³ European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, CB10 1SD, United Kingdom

Corresponding author: Teichmann, SA (st9@sanger.ac.uk)

Current Opinion in Systems Biology 2019, 18:87–94

This review comes from a themed issue on **Systems immunology & host-pathogen interaction**

Edited by **Thomas Höfer** and **Grégoire Altan-Bonnet**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 30 October 2019

<https://doi.org/10.1016/j.coisb.2019.10.005>

2452-3100/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Keywords

Single-cell technologies, Immunity, Cell-cell interactions, Cross-talk applications.

Introduction

Understanding how a complex set of interacting components work together to produce an outcome requires a systems approach. For much of immunology's long history, most insights came from examination of the constituent parts and their individual characteristics and not so much on activities across the whole organism. This is understandable given the complexity of the immune

system with hundreds of antigens, cytokines, chemokines and transcription factors that can be used to define a cell and its function. Single-cell technologies present an important strategy for understanding human immunity in a systematic way and have led to a major change in how research is performed in many laboratories. It is now feasible to use high-throughput methods for an unbiased exploration of a variety of circulating and tissue resident immune cells in health and disease, which has proven to generate new hypotheses. The ability to generate large single-cell atlases has evolved together with bioinformatic tools that enable the user to comprehend the vast amount of data produced [1]. To date, single-cell RNA-sequencing (sc-RNA-seq) has been successful at describing the heterogeneity of the immune system [2,3]. This has led to a discovery of novel circulating and tissue-resident immune cell types and states [4,5], reconstruction of cell lineages [6,7], and their activation status in disease [6,8]. Importantly, it has also revealed unexpected examples of cooperativity and partnerships between cell types [9].

Immune cells constantly survey tissues to monitor their integrity and respond to infections. Unsurprisingly, the greatest activity is found in border organs, including skin, lung and gut, that are all constantly exposed to physical and microbial stresses. In addition, there is a great functional and structural heterogeneity of resident immune cells across tissues. For example, liver macrophages called Kupffer cells specialise in the clearance of aged erythrocytes while gut macrophages regulate immune responses to commensal bacteria [10]. Even though the functional heterogeneity is likely dictated by tissue microenvironment, niche-specific factors involved are largely unknown [10]. That is because each organ is unique and tailored to suit the function of the tissue, containing a combination of specialised cell types from epithelial, endothelial, neural and mesenchymal lineages.

One way to interrogate immunology is by focusing not only on the immune cell types, but also on their non-immune interacting partners, molecules they use for communication and how such cross-talk changes during development, homeostasis and disease. The obvious advantage of single-cell technologies in addressing this task is the ability to sample various cell types in an unbiased way. Using the entire tissue allows not only the study of tissue-resident immune populations and their

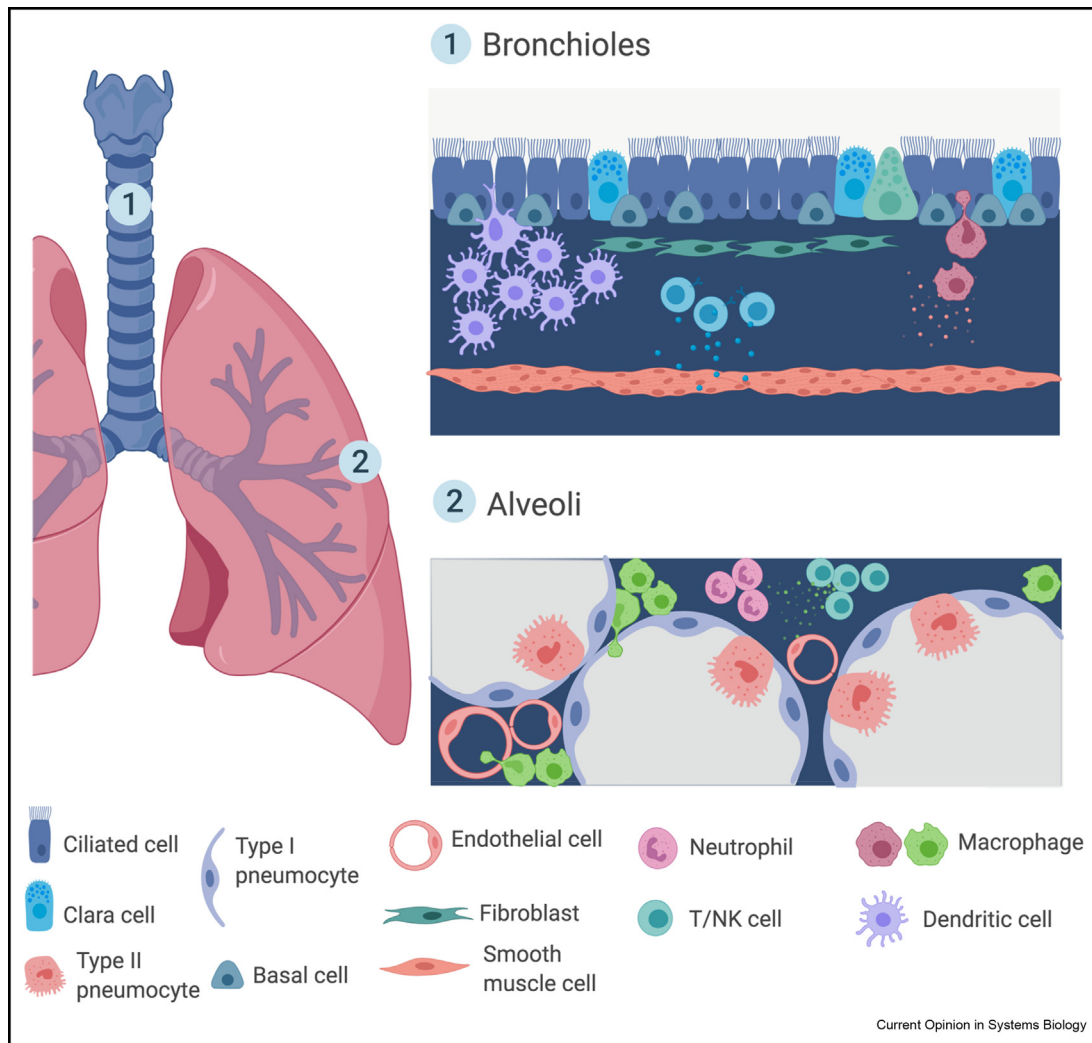
activation or differentiation stages but also their microenvironment. Here, we argue for the need of an integrative view of niche cells to achieve a systems view of immunology. We discuss increasing evidence that tissue cells communicate with resident and circulating immune cells to aid tissue development and regeneration, homeostasis and progression of disease. Finally, we end with a discussion of the existing experimental and computational strategies for dissecting this dialogue using single-cell technologies.

Two sides of a coin: the roles of immune and their niche cells in immunity

Cells can either interact physically via gap junctions and surface receptors or indirectly via secreted paracrine (local) and endocrine (distant) ligands. Predicting cell–

cell communication from transcriptomics data is a challenging task. Nevertheless, multiple studies to date have attempted this task. The most common prediction approach is based on known ligand and receptor pairs and their expression on cells. The analysis requires a number of assumptions including that the receptor transcripts are translated and presented on the membrane, while ligands are successfully transported out of the cells, and are in proximity in tissue space with interacting partners. This is especially relevant to compartmentalised organs, such as lung, where immune and epithelial cell types and their proportions change drastically across multiple locations (Figure 1). Here, we highlight single-cell studies used to comprehensively delineate the functional cross-talk between immune and tissue niche cells and the relevance of these interactions in immunity and human disease.

Figure 1



Compartmentalisation of cell types and interactions within human lung. Single-cell studies can outline the compartmentalisation, cellular content and cell–cell interactions within regions of a single organ. Cell–cell cross-talk will depend on the proportion of cells, proximity between different cell types and availability of interacting partners. Lung is an example of a highly structured organ with two main architectural locations: bronchioles and alveoli. Created with [BioRender.com](https://www.bio-render.com/).

Development and tissue regeneration

Pregnancy presents an exceptional immunological problem, where the mother's immune system interacts with foetal cells in such a way that prevents rejection of 'nonself' and supports foetal growth and development. As shown by us and others, this subtle relationship is governed by the cross-talk between immune and non-immune cell types. An example of such interactions is where early embryonic epithelial trophoblasts express receptors that support maternal–foetal compartmentalisation and block the movement of inflammatory chemokines from the maternal decidua to embryonic circulation [11]. We [9] and others [12,13] have used single-cell technology to comprehensively infer complex interaction patterns during human first trimester and term pregnancies using receptor–ligand pairs. The studies inferred an interplay between maternal natural killer [9] and dendritic cells [13] with embryonic trophoblasts to achieve an anti-inflammatory environment.

The relationship seems to continue throughout adulthood and sustain tissue regeneration in multiple organs. Several studies report a tight cross-talk between tissue stem cells and immune cells as reviewed [14]. Single-cell studies offer unprecedented resolution in comprehensively identifying both the interacting partners and the molecules involved by using transcriptomics data. For example, a subset of T regulatory cells and macrophages was found to interact specifically with hair follicle stem cells [15,16]. Furthermore, both macrophages and ILC2 leukocytes expressing IL-13 were found to promote lung regeneration via modulation of alveolar stem cell self-renewal and differentiation [17]. Macrophages were found to contribute to gastric organoid regeneration *in vitro* [18] and in the murine intestine [19].

Single-cell approaches allow studying cell–cell interaction during mouse and human organogenesis. An example is a study in human foetal intestine, where CD4 Th1-like cells were shown to modulate intestinal growth via interaction with LGR5+ stem cells [20]. The potential of various immune cells including macrophages and basophils to interact with endothelial, fibroblast and epithelial cells was also shown in the developing murine lung [21].

Homeostasis and infection

It is evident that tissue microenvironment changes drastically during infection, inflammation and mechanical injury. Single-cell studies have highlighted the structural and cellular compartmentalisation of various tissues relevant to responses in infections. In skin, fibroblast populations were compartmentalised into anti-inflammatory upper dermis and inflammatory lower dermis, which suggests that upper dermal fibroblasts are

primed to respond to infection more readily [22]. Gene signatures in endothelial venule cells in peripheral lymph nodes [23] and skin fibroblasts [24] were described as consistent with recruitment of naive lymphocytes or retention of inflammatory cells, respectively. In addition, single-cell sequencing of murine lymph nodes has identified nine stromal cell populations that occupy multiple lymph node niches [25]. The study provides evidence that multiple stromal cell types contribute to the compartmentalised microenvironment, are in an activated state in a resting lymph node and guide immune cells during an immune response [25]. Furthermore, a subset of tuft cells from the gut epithelium was found to exhibit an inflammatory gene program with expression of Th2-promoting cytokine and immune cell marker *Ptprc* [26].

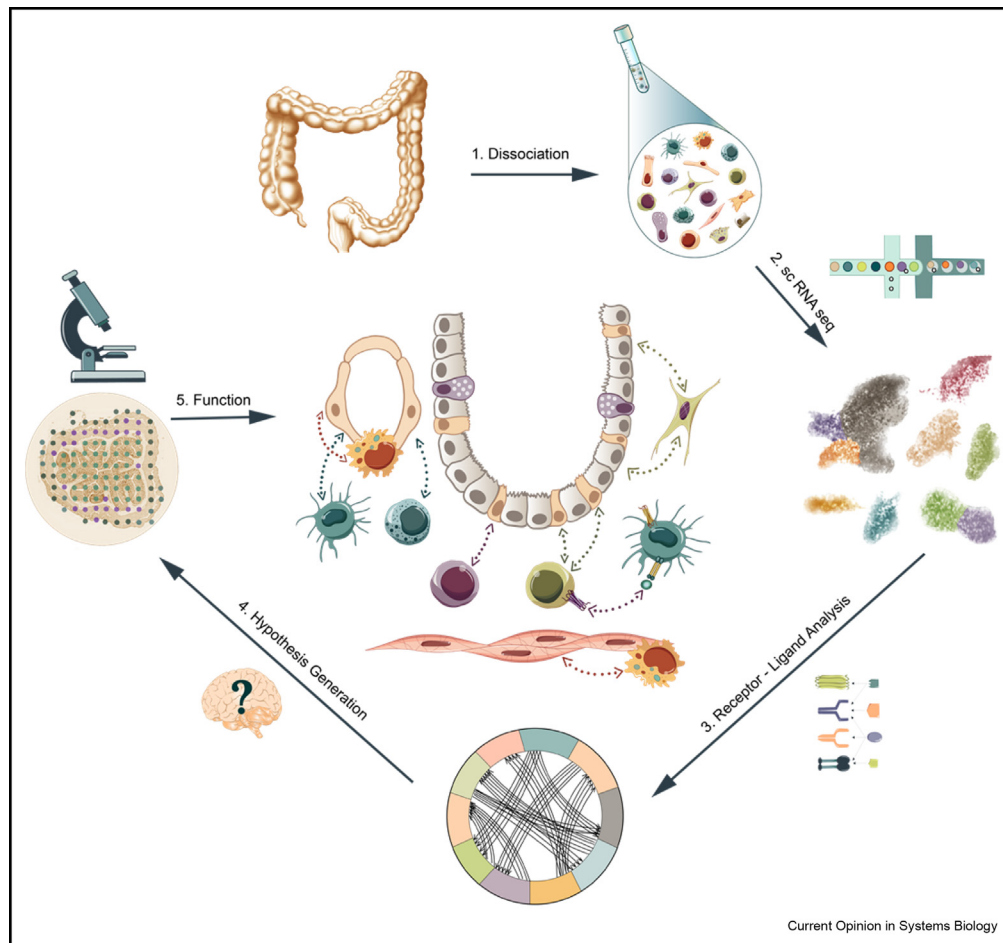
Moreover, the microenvironment can shape the immune cell differentiation potential. A single-cell study by Tikhonova *et al.* [27] described vascular, perivascular and osteoblast cells in the adult bone marrow. They found that Notch ligand *DLL4* expressed by endothelial cells skews differentiation of hematopoietic progenitors to the myeloid lineage. Conversely, a single-cell study of mouse skin during wound healing has identified a subset of myofibroblasts and rare regenerated adipocytes that have originated from myeloid cells [28]. Pseudotime and RNA velocity analyses revealed a subset of contractile fibroblasts that expressed hematopoietic markers and validated that cells originating from the bone marrow give rise to a subset of myofibroblasts and rare regenerated adipocytes during wound healing. Similarly, immune cells can shape epithelial cell differentiation in inflammation. In mice, that has been observed upon helminth and bacterial infection, which results in specialisation of intestinal epithelial cells to different secretory lineages [26,30].

Disease and aging

The immune microenvironment has received a lot of attention in cancer and has been the subject of multiple reviews [31,32]. Single-cell studies have contributed by identifying specific T-cell [33,34] and macrophage [35,36] populations that are predictive of the clinical outcome in lung cancer and melanoma. Furthermore, the spatial distribution of a T-cell subset around the malignant cells was important for the outcome in B-cell lymphoma [37]. These studies outline potential of single-cell profiling tools in both the diagnostics in cancer as well as for development of therapeutics.

Niche cell populations can also shape immune cell function in other human diseases and aging. For example, inflammatory diseases can manifest as a result of imbalanced immune cell recruitment or retention

Figure 2



Schematic workflow for analysing cell–cell interactions. Tissue sample is dissociated and analysed by sc-RNA-seq followed by cell-type identification. Previous knowledge of receptor–ligand pairs is used for inferring cell–cell communication and formulating new hypotheses, which are validated by molecular biology methods, thus providing a model for the interaction network within a sampling location.

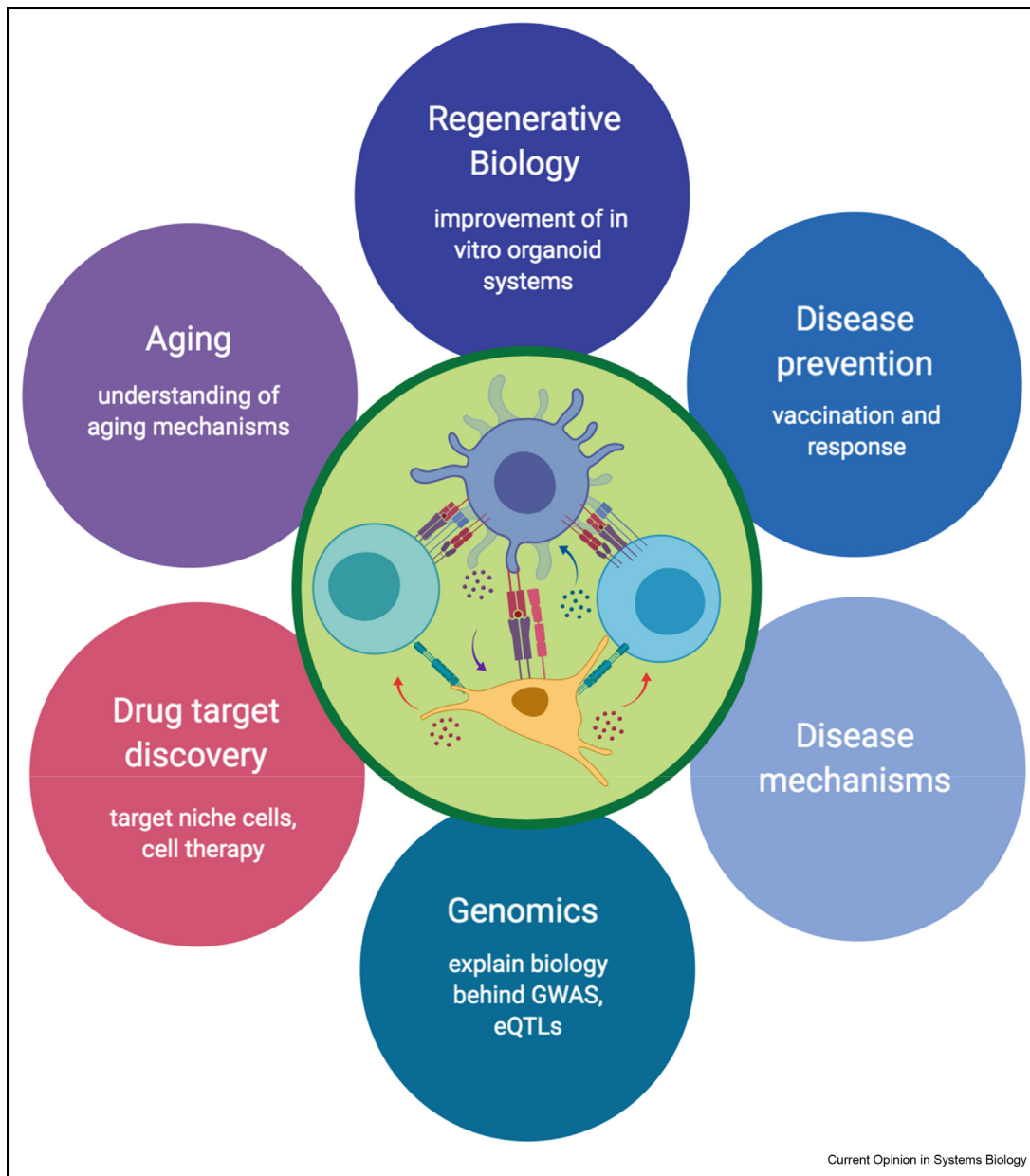
modulated by niche cell signalling. Inflammation-related keratinocyte signatures were enriched in psoriatic skin, alongside increased numbers of a specific *CD1C+CD301A*+myeloid dendritic cell population [38], pointing to a potential recruitment function of niche cells. Also, fibroblasts were associated with recruitment of macrophages [39] and T-cells [40] in melanoma and with retention of T cells in inflamed human skin [41]. Moreover, there has been increased attention on fibroblast populations in inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and cancer. Single-cell study in healthy and IBD patients identified four populations of fibroblast-like cells, one of which was enriched for pro-inflammatory factors and expanded in IBD [42]. Similarly, a population of fibroblasts in the joint sublining layer were associated with severity of inflammation in RA patients [43,44]. Fibroblast invasiveness was further found to be induced via a newly identified subset of macrophages in RA [45].

The relationship between chronic inflammation and aging has been reported and reviewed elsewhere [46,47]; however, the mechanisms are currently unknown. Single-cell studies show higher mutational burden and increasing heterogeneity with age for multiple cell types as well as upregulation of inflammatory signatures both in immune and non-immune cells [48,49]. The combination of having more cytokine-secreting fibroblasts [50] and heterogeneous activation of lymphocytes [50,51] in old mice versus young suggest an increasingly versatile cross-talk between cells in an aging organism. This emphasises the need to study age-related conditions and cell–cell interactions via single-cell methods.

Single-cell strategies for profiling cell–cell communication

To use sc-RNA-seq data to comprehensively map all immune to non-immune cell interactions, there is a need to optimise the unbiased capture of all cell types

Figure 3



Applications of cell–cell interaction networks in science and medicine. Studies of interaction networks in the single-cell resolution will advance both basic and applied research. Better understanding of regenerative biology, aging and genetic vs environment relationship will lead to better cell therapies and discovery of new drug targets. Created with BioRender.com.

in single-cell experiments. Indeed, the unbiased sampling of organs to date has highlighted previously unappreciated diversity in immune and non-immune cell types in healthy human lung [8,52], liver [53], colon [42,54], skin [38,55] and pancreas [56,57]. However, the unbiased sampling requires sequencing of large numbers of cells. This increases the cost of a study, especially if one major cell type dominates over others. Also, not all cell types respond equally to the same

sample processing protocol, with some cell types requiring harsh dissociation methods that are likely to kill other cell types. For example, structural parts of a tissue, like smooth muscle cells or enteric neuron networks in the gut, will require longer and harsher dissociation than epithelial cells of the lamina propria. Therefore, the same tissue might have to be sampled using different dissociation protocols to cover the diversity of cell types present.

To down-sample abundant cell types, enrichment of cell lineages can be done to ensure balanced cell capture. For example, less abundant populations can be sorted using a marker gene, such as immune (CD45RA), epithelial (EPCAM) or fibroblast (COL1A1) cells, which can be combined with the remaining cells in desired proportions [9,40,58]. This approach leads to datasets with better balance of different cell types and reduction in sequencing costs. However, the downsides include a longer manipulation time for cell enrichment that might affect cell viability or induce stress response gene expression [59]. Finally, it may also be of interest to interrogate the signalling of two known, but rare interacting partners. In this case, classical isolation and in-depth sequencing of underrepresented cell population could be used combined with unbiased sampling. To date, the combination of single-cell methods with other approaches have predicted cell–cell proximity such as using partial dissociation and downstream deconvolution of the signal [60] or cell lineage labelling methods such as NICHE-seq [61].

Outlook and summary

In single-cell experimental design, there is a trade-off between in-depth profiling of specific cell types and unbiased mapping of all cell types present in the tissue. Apart from describing immune heterogeneity, it has provided a systems insight into immunology during development, regeneration, infection and disease.

As evident from the studies discussed above, single-cell methods alone can be extremely informative in providing cell–cell interaction snapshots in human tissues (Figure 2). The publicly available receptor–ligand inference tools such as CellPhoneDB [9] and ProximID [60] are user friendly and easily applicable. However, major disadvantages of sc-RNA-seq include loss of spatial information including cell–cell proximity as well as the distribution of signal within a cell, especially in highly structured cell types such as neurons and dendritic cells. Therefore, there is a need for high-resolution spatial methods that can capture the full transcriptome *in situ*. Although traditional microscopy, with either use of antibodies [37] or RNA probes [62], provides subcellular resolution, a very limited set of features per cell can be studied. High-throughput spatial genomics methods aim to characterise gene expression from single cells while retaining information on tissue context. The methods of hybridisation by MERFISH [63] or sequencing by FISSEQ [64] on slides captures expression of up to thousands of genes and their subcellular locations. Although Spatial Transcriptomics methods [65] allow for the complete transcriptomic profile of a tissue region containing a dozen to hundreds of cells, a higher density array such as Slide-seq [63] allows for subcellular localisation with up to multiple cellular localisations captured.

Currently, none of these methods match sc-RNA-seq in price, accuracy, depth, robustness and number of cells gained. However, we anticipate that if the methods combining spatial subcellular resolution and transcriptomics improve, they will be very informative and widely used in the future.

As the Human Cell Atlas (www.humancellatlas.org) initiative is working on describing all cell types in the human body, the meta-analysis of cell–cell interaction networks in multiple tissues will provide another level of insight, including the inference of paracrine signalling. In the future, deconvolution of interactions using multi-omics including genomics, proteomics, metabolomics and gene sequencing data will be the key to better understand the downstream signalling and correlations among genes, proteins and lipids at the single-cell level. As the repository of all possible interactions and downstream signalling increases, so will the ability to interpret the interactions between single cells.

Better understanding of cell–cell interactions will inevitably lead to multiple applications (Figure 3) [66]. For example, culture conditions can be inferred from newly defined signalling pathways. This will have applications in improving *in vitro* differentiation processes and regenerative biology [67]. Cellular identity of the interacting partners in disease will provide new candidates for cell therapies and enhance the effectiveness of existing ones [8,68]. Furthermore, interactions may aid in understanding and predicting tissue and cell type specific efficacy of drugs and vaccinations as well as their side-effects. With more data becoming available, the ability to explain environmental and genetic effects on cancer, drug response, chronic inflammation and others will become a reality.

Conflict of interest statement

Nothing declared.

Acknowledgements

We would like to thank Mirjana Efremova, Tomas Gomes, Marius Rebmann and Kylie James for discussions, Sarah Aldridge for proofreading the manuscript, and Jana Eliasova for graphically designing Figure 2. This work is supported by Wellcome Sanger core funding (WT206194) and Chan Zuckerberg Foundation grant number: 2017-174169 (5022).

References

Papers of particular interest, published within the period of review, have been highlighted as:

- * of special interest
 - ** of outstanding interest
1. Angerer P, Simon L, Tritschler S, Alexander Wolf F, Fischer D, Theis FJ: **Single cells make big data: new challenges and opportunities in transcriptomics**. *Internet Curr Opin Struct Biol* 2017:85–91, <https://doi.org/10.1016/j.coisb.2017.07.004>.
 2. Chen H, Ye F, Guo G: **Revolutionizing immunology with single-cell RNA sequencing**. *Cell Mol Immunol* 2019, **16**: 242–249.

3. Papalexis E, Satija R: **Single-cell RNA sequencing to explore immune cell heterogeneity.** Internet *Nat Rev Immunol* 2018; 35–45, <https://doi.org/10.1038/nri.2017.76>.
4. Villani A-C, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, *et al.*: **Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors.** *Science* 2017, **356**, <https://doi.org/10.1126/science.aah4573>.
Discovered novel dendritic and monocyte populations in human blood using sorting and sequencing of rare populations.
5. Crinier A, Milpied P, Escalière B, Piperoglou C, Galluso J, Balsamo A, *et al.*: **High-Dimensional single-cell analysis identifies organ-specific signatures and conserved NK cell subsets in humans and mice.** *Immunity* 2018, **49**:971–986. e5.
6. Zheng C, Zheng L, Yoo J-K, Guo H, Zhang Y, Guo X, *et al.*: **Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing.** *Cell* 2017, **169**:1342–1356. e16.
7. See P, Dutertre C-A, Chen J, Günther P, McGovern N, Irac SE, *et al.*: **Mapping the human DC lineage through the integration of high-dimensional techniques.** *Science* 2017, **356**, <https://doi.org/10.1126/science.aag3009>.
8. Braga FAV, Kar G, Berg M, Carpaij OA, Polanski K, Simon LM, *et al.*: **A cellular census of human lungs identifies novel cell states in health and in asthma.** *Nat Med* 2019, **25**:1153–1163.
Cellular composition of the human airways show differences in epithelial, stromal and immune cell proportions between different regions, as well as between healthy and asthmatic lung.
9. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, *et al.*: **Single-cell reconstruction of the early maternal-fetal interface in humans.** *Nature* 2018, **563**:347–353.
Developed receptor-ligand inference tool, called CellPhoneDB, and applied it to comprehensively map cell-type communication networks across maternal and fetal tissues during pregnancy.
10. Davies LC, Jenkins SJ, Allen JE, Taylor PR: **Tissue-resident macrophages.** *Nat Immunol* 2013, **14**:986–995.
11. Lee KM, Wilson GJ, Pingen M, Fukuoka A, Hansell CAH, Bartolini R, *et al.*: **Placental chemokine compartmentalisation: a novel mammalian molecular control mechanism.** *PLoS Biol* 2019, **17**, e3000287.
12. Suryawanshi H, Morozov P, Straus A, Sahasrabudhe N, Max KEA, Garzia A, *et al.*: **A single-cell survey of the human first-trimester placenta and decidua.** *Sci Adv* 2018, **4**, eaau4788.
13. Pavličev M, Wagner GP, Chavan AR, Owens K, Maziarz J, Dunn-Fletcher C, *et al.*: **Single-cell transcriptomics of the human placenta: inferring the cell communication network of the maternal-fetal interface.** *Genome Res* 2017, **27**:349–361.
14. Naik S, Larsen SB, Cowley CJ, Fuchs E: **Two to tango: dialog between immunity and stem cells in health and disease.** *Cell* 2018, **175**:908–920.
15. Ali N, Zirik B, Rodriguez RS, Pauli ML, Truong H-A, Lai K, *et al.*: **Regulatory T cells in skin facilitate epithelial stem cell differentiation.** *Cell* 2017, **169**:1119–1129. e11.
16. Wang ECE, Dai Z, Ferrante AW, Drake CG, Christiano AM: **A subset of TREM2 dermal macrophages secretes oncostatin M to maintain hair follicle stem cell quiescence and inhibit hair growth.** *Cell Stem Cell* 2019, **24**:654–669. e6.
17. Lechner AJ, Driver IH, Lee J, Conroy CM, Nagle A, Locksley RM, *et al.*: **Recruited monocytes and type 2 immunity promote lung regeneration following pneumectomy.** *Cell Stem Cell* 2017, **21**:120–134. e7.
18. Chen J, Lau BT, Andor N, Grimes SM, Handy C, Wood-Bouwens C, *et al.*: **Single-cell transcriptome analysis identifies distinct cell types and niche signaling in a primary gastric organoid model [Internet].** *Sci Rep* 2019, <https://doi.org/10.1038/s41598-019-40809-x>.
19. Sehgal A, Donaldson DS, Pridans C, Sauter KA, Hume DA, Mabbott NA: **The role of CSF1R-dependent macrophages in control of the intestinal stem-cell niche.** *Nat Commun* 2018, **9**:1272.
20. Schreurs RRCE, Baumdick ME, Sagebiel AF, Kaufmann M, Mokry M, Klarenbeek PL, *et al.*: **Human fetal TNF- α -cytokine-producing CD4 effector memory T cells promote intestinal development and mediate inflammation early in life.** *Immunity* 2019, **50**:462–476. e8.
21. Cohen M, Giladi A, Gorki A-D, Solodkin DG, Zada M, Hladik A, *et al.*: **Lung single-cell signaling interaction map reveals basophil role in macrophage imprinting.** *Cell* 2018, **175**:1031–1044. e18.
22. Philippes C, Telerman SB, Oulès B, Pisco AO, Shaw TJ, Elgueta R, *et al.*: **Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations.** *J Invest Dermatol* 2018, **138**:811–825.
23. Veerman K, Tardiveau C, Martins F, Coudert J, Girard J-P: **Single-cell analysis reveals heterogeneity of high endothelial venules and different regulation of genes controlling lymphocyte entry to lymph nodes [Internet].** *Cell Rep* 2019: 3116–3131, <https://doi.org/10.1016/j.celrep.2019.02.042>. e5.
24. Tabib T, Morse C, Wang T, Chen W, Lafyatis R: **SFRP2/DPP4 and FMO1/LSP1 define major fibroblast populations in human skin.** *J Invest Dermatol* 2018, **138**:802–810.
25. Rodda LB, Lu E, Bennett ML, Sokol CL, Wang X, Luther SA, *et al.*: **Single-cell RNA sequencing of lymph node stromal cells reveals niche-associated heterogeneity.** *Immunity* 2018, **48**:1014–1028. e6.
26. Haber AL, Biton M, Rogel N, Herbst RH, Shekhar K, Smillie C, *et al.*: **A single-cell survey of the small intestinal epithelium.** *Nature* 2017, **551**:333–339.
27. Tikhonova AN, Dolgalev I, Hu H, Sivaraj KK, Hoxha E, Cuesta-Dominguez Á, *et al.*: **The bone marrow microenvironment at single-cell resolution.** *Nature* 2019, **569**:222–228.
28. Guerrero-Juarez CF, Dedhia PH, Jin S, Ruiz-Vega R, Ma D, Liu Y, *et al.*: **Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds.** *Nat Commun* 2019, **10**:650.
30. Biton M, Haber AL, Rogel N, Burgin G, Beyaz S, Schnell A, *et al.*: **T helper cell cytokines modulate intestinal stem cell renewal and differentiation.** Internet *Cell* 2018:1307–1320, <https://doi.org/10.1016/j.cell.2018.10.008>. e22.
Uses single-cell RNA-sequencing to show the T helper cell subtype functional ability to remodel intestinal epithelium in adult mouse.
31. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, *et al.*: **Understanding the tumor immune microenvironment (TIME) for effective therapy.** *Nat Med* 2018, **24**:541–550.
32. Taube JM, Galon J, Sholl LM, Rodig SJ, Cottrell TR, Giraldo NA, *et al.*: **Implications of the tumor immune microenvironment for staging and therapeutics.** *Mod Pathol* 2018, **31**:214–234.
33. Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, *et al.*: **Defining T cell states associated with response to checkpoint immunotherapy in melanoma.** *Cell* 2019, **176**:404.
34. Guo X, Zhang Y, Zheng L, Zheng C, Song J, Zhang Q, *et al.*: **Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing.** *Nat Med* 2018, **24**:978–985.
35. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, *et al.*: **Phenotype molding of stromal cells in the lung tumor microenvironment.** *Nat Med* 2018, **24**:1277–1289.
36. Venteicher AS, Tirosh I, Hebert C, Yizhak K, Neftci C, Filbin MG, *et al.*: **Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq.** *Science* 2017, **355**, <https://doi.org/10.1126/science.aai8478>.
37. Xu-Monette ZY, Xiao M, Au Q, Padmanabhan R, Xu B, Hoe N, *et al.*: **Immune profiling and quantitative analysis decipher the clinical role of immune-checkpoint expression in the tumor immune microenvironment of DLBCL.** *Cancer Immunol Res* 2019, **7**:644–657.
Demonstration of diagnostic power when combining spatial and gene expression data in malignant and immune cells expressing PD-1 and its ligands.

38. Cheng JB, Sedgewick AJ, Finnegan AI, Harirchian P, Lee J, Kwon S, *et al.*: **Transcriptional programming of normal and inflamed human epidermis at single-cell resolution.** *Cell Rep* 2018, **25**:871–883.
39. Davidson S, Efremova M, Riedel A, Mahata B, Pramanik J, Huuhtanen J, *et al.*: Single-cell RNA sequencing reveals a dynamic stromal niche within the evolving tumour microenvironment [Internet]. doi:10.1101/467225.
40. Tirosh I, Izar B, Prakadan SM, Wadsworth 2nd MH, Treacy D, Trombetta JJ, *et al.*: **Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq.** *Science* 2016, **352**:189–196.
41. Barron AMS, Mantero JC, Ho JD, Nazari B, Horback KL, Bhawan J, *et al.*: **Perivascular adventitial fibroblast specialization accompanies T cell retention in the inflamed human dermis.** *J Immunol* 2019, **202**:56–68.
42. Kinchen J, Chen HH, Parikh K, Antanaviciute A, Jagielowicz M, Fawcner-Corbett D, *et al.*: **Structural remodeling of the human colonic mesenchyme in inflammatory bowel disease.** *Cell* 2018, **175**:372–386. e17.
43. Croft AP, Campos J, Jansen K, Turner JD, Marshall J, Attar M, *et al.*: **Distinct fibroblast subsets drive inflammation and damage in arthritis.** *Nature* 2019, <https://doi.org/10.1038/s41586-019-1263-7>.
44. Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S, *et al.*: **Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry.** *Nat Immunol* 2019, <https://doi.org/10.1038/s41590-019-0378-1>.
45. Kuo D, Ding J, Cohn IS, Zhang F, Wei K, Rao DA, *et al.*: **HBEGF macrophages in rheumatoid arthritis induce fibroblast invasiveness.** *Sci Transl Med* 2019, **11**, <https://doi.org/10.1126/scitranslmed.aau8587>.
46. Franceschi C, Campisi J: **Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases.** *J Gerontol A Biol Sci Med Sci* 2014, **69**(Suppl 1):S4–S9.
47. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A: **Inflammaging: a new immune-metabolic viewpoint for age-related diseases.** *Nat Rev Endocrinol* 2018, **14**:576–590.
48. Kimmel JC, Penland L, Rubinstein ND, Hendrickson DG, Kelley DR, Rosenthal AZ. A murine aging cell atlas reveals cell identity and tissue-specific trajectories of aging [Internet]. doi:10.1101/657726.
49. Consortium TTM, The Tabula Muris consortium, Pisco AO, Schaum N, McGeever A, Karkanas J, *et al.*: A single cell transcriptomic atlas characterizes aging tissues in the mouse [Internet]. doi:10.1101/661728.
50. Mahmoudi S, Mancini E, Moore A, Xu L, Jahanbani F, Hebestreit K, *et al.*: Old fibroblasts secrete inflammatory cytokines that drive variability in reprogramming efficiency and may affect wound healing between old individuals [Internet]. doi:10.1101/448431.
51. Martinez-Jimenez CP, Eling N, Chen H-C, Vallejos CA, Kolodziejczyk AA, Connor F, *et al.*: **Aging increases cell-to-cell transcriptional variability upon immune stimulation.** *Science* 2017, **355**:1433–1436.
52. Plasschaert LW, Žiljionis R, Choo-Wing R, Savova V, Knehr J, Roma G, *et al.*: **A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte.** *Nature* 2018, **560**:377–381.
- Profiling of the cell types in the human and mouse airway epithelial cells discover a rare pulmonary ionocyte population that expresses the cystic fibrosis gene CFTR.
53. MacParland SA, Liu JC, Ma X-Z, Innes BT, Bartczak AM, Gage BK, *et al.*: **Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations.** *Nat Commun* 2018, **9**:4383.
54. Parikh K, Antanaviciute A, Fawcner-Corbett D, Jagielowicz M, Alicino A, Lagerholm C, *et al.*: **Colonic epithelial cell diversity in health and inflammatory bowel disease.** *Nature* 2019, **567**:49–55.
55. Solé-Boldo L, Raddatz G, Schütz S, Mallm J-P, Rippe K, Lonsdorf AS, *et al.*: Single-cell transcriptomes of the aging human skin reveal loss of fibroblast priming [Internet]. doi:10.1101/633131.
56. Muraro MJ, Dharmadhikari G, Grün D, Groen N, Dielen T, Jansen E, *et al.*: **A single-cell transcriptome atlas of the human pancreas.** *Cell Syst* 2016, **3**:385–394. e3.
57. Enge M, Arda HE, Mignardi M, Beausang J, Bottino R, Kim SK, *et al.*: **Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns.** *Cell* 2017, **171**:321–330. e14.
58. Goh M, Lim ZM, Koh V, Lum J, Zhang X, McGovern N, *et al.*: **Single-cell analysis of immune-microenvironment and immune-tumor interaction in human gastric cancers** [Internet] *J Clin Oncol* 2019, https://doi.org/10.1200/jco.2019.37.4_suppl.29. 29–29.
59. van den Brink SC, Sage F, Vértesy Á, Spanjaard B, Peterson-Maduro J, Baron CS, *et al.*: **Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations.** *Nat Methods* 2017, **14**:935–936.
60. Boisset J-C, Vivié J, Grün D, Muraro MJ, Lyubimova A, van Oudenaarden A: **Mapping the physical network of cellular interactions.** *Nat Methods* 2018, **15**:547–553.
- Developed ProximID tool for analysing cell–cell interactions from the single-cell RNA-sequencing data.
61. Medaglia C, Giladi A, Stoler-Barak L, De Giovanni M, Salame TM, Biram A, *et al.*: **Spatial reconstruction of immune niches by combining photoactivatable reporters and scRNA-seq.** *Science* 2017, **358**:1622–1626.
62. Lubeck E, Coskun AF, Zhiyentayev T, Ahmad M, Cai L: **Single-cell in situ RNA profiling by sequential hybridization.** *Nat Methods* 2014, **11**:360–361.
63. Rodrigues SG, Stickels RR, Goeva A, Martin CA, Murray E, Vanderburg CR, *et al.*: **Slide-seq: a scalable technology for measuring genome-wide expression at high spatial resolution.** *Science* 2019, **363**:1463–1467.
64. Lee JH, Daugharthy ER, Scheiman J, Kalhor R, Ferrante TC, Terry R, *et al.*: **Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues.** *Nat Protoc* 2015, **10**:442–458.
65. Ståhl PL, Salmén F, Vickovic S, Lundmark A, Navarro JF, Magnusson J, *et al.*: **Visualization and analysis of gene expression in tissue sections by spatial transcriptomics.** *Science* 2016, **353**:78–82.
66. Regev A, Teichmann S, Rozenblatt-Rosen O, Stubbington M, Ardlie K, Amit I, *et al.*: **The Human Cell Atlas White Paper.** *arXiv* 11 Oct 2018. <https://arxiv.org/abs/1810.05192>; 11 Oct 2018.
67. Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, *et al.*: **Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition.** *Cell Stem Cell* 6 Dec 2018, **23**:787–793. e6.
68. Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, *et al.*: **Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis.** *Cell* 25 July 2019, **178**:714–730. e22.