Uncovering intestinal macrophages through the integration of single-cell and spatial transcriptomics

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Single-cell RNA sequencing (scRNA-seq) has expanded our understanding of a tissue's cellular composition and provided the biomedical field with an invaluable tool to map disease-associated patterns at a resolution previously unattainable[1]. Furthermore, single-cell transcriptomics has enabled the discovery of dynamic and transitional cell states, challenging the traditional notion of discrete cell populations. One of the revelations of applying single-cell transcriptomics to tissue analysis has been the degree of heterogeneity that exists within cell lineages in both health and disease. Nonetheless, scRNA-seq has also brought with it certain challenges, including the difficulty of achieving consensus in cell type annotations, both within the same tissue and across different tissues, which can limit cross-study validation. In addition, the interpretation of scRNA-seq data has limitations; while the transcriptional profile of a cell reflects its lineage and state, it may not be enough to infer its function in a specific tissue or disease context.

To overcome some of these weaknesses, one strategy involves combining single-cell transcriptomics data with proteomics or epigenomics analysis, which could help better characterize cell identities and infer cellular functions[2]. More recently, spatial transcriptomics methods that measure gene expression at large scale, some of them at single-cell resolution, on tissue slides have become increasingly available[3]. Compared to scRNA-seq data, which is generated from cell suspensions after tissue digestion, spatial transcriptomics maps the expression of at least one hundred genes across tissue structures, potentially helping us better understand a cell's function. In a recent publication from our lab[4], we applied spatial transcriptomics at single-cell resolution to study cellular diversity in the colon of inflammatory bowel disease (IBD) patients (Figure 1). In brief, scRNA-seq identified a previously undescribed subset of resident macrophages (M0) that co-exist with classical M2 macrophages in the healthy and inflamed colon. Remarkably, colonic

inflammation was associated with the expansion of the macrophage compartment which, besides the resident populations, included abundant macrophages in activated states ranging from alternative to full-blown M1 macrophages. Within activated macrophage states, we found a novel cluster of cells that expressed *NRG1*, among other genes, and that we annotated as Inflammation-Dependent Alternative (IDA) macrophages. These were present exclusively in inflamed colonic samples, but lacked expression of classical M1 markers (*ACOD1*, *CXCL5*, *TNIP3*, *IL1B*, *INHBA*). While IDA macrophages showed a distinct transcriptional signature, their function and tissue location were unknown. As with other cell types that have been described using scRNA-seq, IDA macrophages lacked unique surface markers that could support their identification and/or their isolation from tissues, limiting our ability to study them. By applying spatial transcriptomics, however, we localized M0, M2, M1 and IDA macrophages within tissue sections of patients suffering from IBD. In brief, we used a panel containing around 1000 genes, and transferred the annotations (labels) that were generated by scRNAseq onto the spatial transcriptomics data. This approach provided a powerful tool to map all cell types found in tissue sections including all epithelial, lymphoid, and stromal subsets. Beyond validating the existence of two types of resident macrophages and diverse activated ones in IBD, spatial analysis shows us that cells annotated as one cluster may be present at diverse tissue locations. Thus, they may potentially represent a more heterogeneous class than anticipated by scRNA-seq analysis. Indeed, spatial analysis identified macrophages with an IDA signature in at least two distinct tissue structures, which could suggest different functions. Further supporting this view, we found that IDA macrophages located beneath the surface epithelium expressed high levels of *NRG1*, which encodes for neuregulin-1, a ligand of the EGF receptor that promotes epithelial expression of olfactomedin-4. In contrast, IDA macrophages within granuloma formations in a patient

suffering from Crohn's disease (one of the two main forms of IBDs) lacked *NRG1* expression. In addition, the use of spatial transcriptomics compared to scRNA-seq allowed us to find spatial correlations between different cell types. For instance, we found that IDA macrophages localized near other macrophage subsets (M0, M1 and M2) and several subsets of T cells within granulomas. Within the stromal compartment, IDA macrophages showed a high spatial correlation with inflammatory fibroblasts, a population of fibroblasts previously observed in the inflamed intestine of IBD patients[5].

Many possibilities remain ahead. For instance, spatial transcriptomics data could not only potentially support the identification of tissue neighborhoods with likely pathological implications, but also reveal key cell-to-cell and receptor-to-ligand interactions that could drive the development of novel therapeutic targets. Both applications require the development of the appropriate computational tools, some of which are now becoming available. In addition, spatial transcriptomics (especially when gene panels are custom made by users based on the specificities of the tissue at hand) can support direct cellular annotation, which could yield spatial information for further annotation of cell types. Such progress will provide an additional layer of information, potentially facilitating a consensus on their annotations.

In summary, we believe that the combination of scRNA-seq and spatial transcriptomics can provide a comprehensive view of cellular biology, tissue organization, and disease processes, making it a powerful tool across different fields of research. Moreover, the use of both techniques may have significant implications for clinical research and personalized medicine, as a combined approach could help identify specific cell populations or regions of interest in the tissue, thereby aiding the development of new diagnostic and treatment strategies (Figure 1).

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FIGURE LEGEND

Figure 1. Integration of single-cell RNA sequencing with cutting-edge spatial transcriptomics can provide a better understanding of cell types and cell functions in both healthy and diseased tissues. This innovative approach helps decipher the cellular and molecular complexity of biologically relevant tissue structures and holds the potential to revolutionize clinical outcomes.

ADDITIONAL INFORMATION

Conflict of Interest: not applicable

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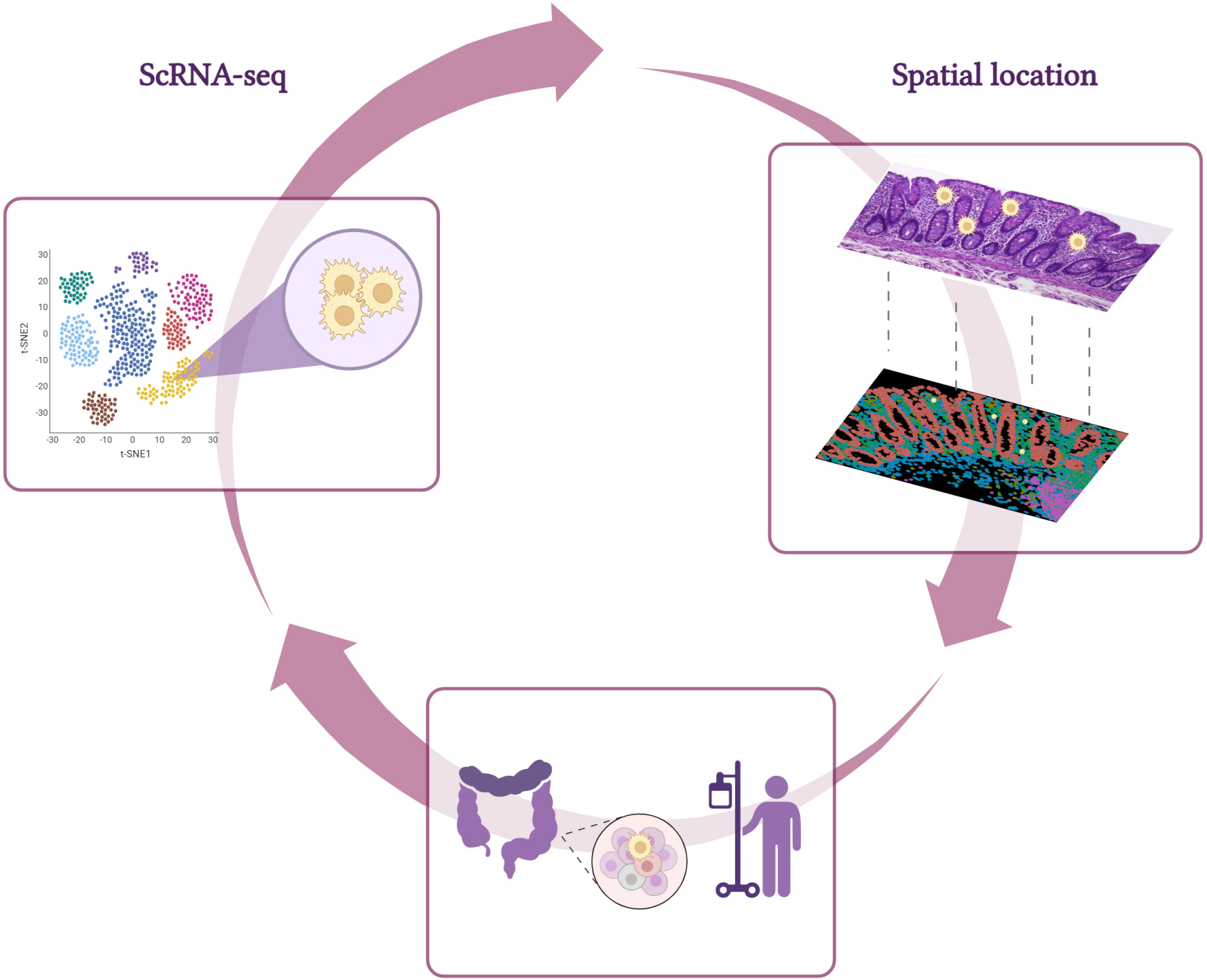
ScRNA-seq
Spatial location
Clinical relevance
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