The Use of Single-Cell RNA-Sequencing and Spatial Transcriptomics in Understanding the Pathogenesis and Treatment of Skin Diseases

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The development of multiomic profiling tools has rapidly expanded in recent years, along with their use in profiling skin tissues in various contexts, including dermatologic diseases. Among these tools, single-cell RNA-sequencing (scRNA-seq) and spatial transcriptomics (ST) have emerged as widely adopted and powerful assays for elucidating key cellular components and their spatial arrangement within skin disease. In this paper, we review the recent biological insights gained from the use of scRNA-seq and ST and the advantages of combining both for profiling skin diseases, including aberrant wound healing, inflammatory skin diseases, and cancer. We discuss the role of scRNA-seq and ST in improving skin disease treatments and moving toward the goal of achieving precision medicine in dermatology, whereby patients can be optimally matched to treatments that maximize therapeutic response.

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INTRODUCTION

Precision medicine has been heralded as a promising avenue for improving clinical practices. In this approach, a patient's individual characteristics, including those related to genetics, environment, and lifestyle, are used to inform clinical decision making. Central to these efforts are big data acquisition and analysis, which use all available patient information to stratify individuals into groups that can be used for prediction of events such as treatment response, disease progression rates, and the likelihood of disease recurrence (Ashley, 2016). Although a significant amount of attention has been paid to patient genotyping, recent novel technologies enable the procurement of high-resolution spatiotemporal and molecular data through direct patient tissue profiling, adding unique insights into our understanding of patient disease. Technologies such as single-cell RNA-sequencing (scRNA-seq) and spatial transcriptomics (ST) are two examples of such innovations (Piñeiro et al., 2022; Wu et al., 2018). scRNA-seq provides a transcriptome-wide readout of individual cells within a tissue sample, enabling new and unbiased insights into tissue heterogeneity. However, inherent limitations of scRNA-seq due to tissue dissociation lose spatial information and introduce the possibility of unintended gene expression changes in response to handling steps. ST, conversely, allows for transcriptomic measurements on intact tissue, thus retaining the spatial architecture, enabling enhanced analyses that infer cell–cell interactions (CCIs). However, ST technologies at large suffer from limitations in the maximal transcript capture efficiency, throughput, or resolution of transcript locality across all modalities (Table 1). Combining these technologies therefore leverages each technology's advantages, optimizing the potential to coordinate high-resolution transcriptomic profiling in spatial contexts.

Given the accessibility of human skin tissue, it is not surprising that these techniques have been readily adopted for profiling a wide range of dermatologic conditions, elucidating the contribution of subpopulations in CCI networks, active molecular signaling pathways, and spatiotemporal stages of both healthy and diseased skin (Figure 1). From a research perspective, these data enable novel hypothesis generation and mechanistic dissection when applied to disease models or distinct biological contexts. From a clinical perspective, this knowledge is particularly useful for the designation of diagnostic biomarkers, identification of novel therapeutic targets, and improving prognostication efforts. Development of multiomic tools, all steadily approaching single-cell resolution, and applying them to disease is comparable with acquiring a new piece of a constantly evolving puzzle. Assembling these pieces will ultimately expand the prospects of precision medicine by realizing the potential to robustly connect clinical phenomena to empirical measurements. Although several previous reviews cover the application of scRNA-seq (Dubois et al., 2021; Wu et al., 2018) or ST (Piñeiro et al., 2022) for studying skin, this article focuses on how combining scRNA-seq and ST profiling on skin

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Abbreviations: AD, atopic dermatitis; ATAC-seq, assay of transposase-accessible chromatin with sequencing; CAF, cancer-associated fibroblast; CCI, cell–cell interaction; CLE, cutaneous lupus erythematosus; ERK, extracellular signal–regulated kinase; GEP, gene expression profiling; HIF1α, hypoxia-inducible factor 1α; IHC, immunohistochemistry; KC, keratinocyte; L-lep, lepromatous leprosy; NCSC, neural crest stem cell; PDX, patient-derived xenograft; PV, psoriasis vulgaris; RR, reversal reaction; scRNA-seq, single-cell RNA-sequencing; ST, spatial transcriptomics; Th, T helper; T-lep, tuberculoid leprosy; TME, tumor microenvironment; Trm1, tissue-resident memory T; TSK, tumor-specific keratinocyte

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Table 1. Overview of Available Commercialized Spatial Transcriptomics Platforms

Commercialized Assay	Resolution	Capture Method	Number of Gene Targets	Advantages	Limitations
Vizgen, MERFISH	Subcellular	Targeted	500	Subcellular resolution High RNA capture efficiency Multimodal data with up to five protein targets simultaneously Custom gene panels	Requires specialized equipment Resources and labor scale with number of readouts Destructive analysis
10X, Visium	55 μM diameter capture spots	Unbiased	Whole transcriptome through NGS	Unbiased readout Requires minimal specialized equipment	Low capture efficiency Low resolution
10X, Xenium	Subcellular	Targeted	~400	Subcellular resolution Nondestructive analysis Large imaging area (2.88 cm^2) Increased throughput of scanning two slides simultaneously	Limited readout Requires specialized equipment
nanoString, CosMx	Subcellular	Targeted	1,000	Multimodal data (can capture up to 64 protein analytes) Subcellular resolution	Requires specialized equipment
nanoString, GeoMx	0–700 microns Single-cell	Targeted and Unbiased	96 targets with nCounter Readout or whole transcriptome through NGS	Multiome data Nondestructive analysis	Requires specialized equipment Need to select region of interest
Spatial Genomics, seqFish	Subcellular	Targeted	Up to 249	Subcellular resolution High RNA capture efficiency	Samples are sent directly to centralized processing center Readout limited to RNA targets
Curio Biosciences, Curio Seeker	1–2 cell resolution (10 μm spatially indexed beads)	Unbiased	Whole transcriptome	Requires minimal specialized equipment	Limited user experience currently Low capture efficiency
Bio-Techne, RNAscope HiPlex v2	Subcellular	Targeted	12 targets (FFPE) and 48 targets (fresh or fixed frozen)	Subcellular resolution Highly validated underlying technology	Highly limited readout

Abbreviations: FFPE, formalin-fixed paraffin-embedded; NGS, next-generation sequencing.

diseases is driving new insights into disease pathogenesis and treatments in addition to serving as potential clinical tools. We discuss current translational limitations and the most practical steps forward for achieving the ultimate goal of precision dermatology.

DISEASE PATHOGENESIS INSIGHTS FROM INTEGRATION OF scRNA-SEQ AND ST

Wound healing

The mechanisms underlying the canonical stages of inflammation, proliferation, and remodeling remain elusive. Efforts to define these dynamics are necessary for understanding normal repair processes and instances when disruption to wound healing occurs. In these cases, scRNA-seq and ST have provided unique insights. In mouse models, temporal studies have more closely characterized the transcriptional and spatial dynamics of wound-associated fibroblast subpopulations throughout the phases of cutaneous wound healing (Foster et al., 2021). In these events, wound healing fibroblasts are first activated after chromatin changes associated with mechanical signaling, and transcriptional activation and cell proliferation occur thereafter. Along a 2-week postwounding timeframe, four distinct transcriptional phenotypes of wound healing fibroblasts localize to the wound: mechanofibrotic, activated responder, proliferator, and remodeling. Multimodal analyses revealed that these subpopulations are distinguishable by their divergent transcriptional and spatial associations within the inner and outer wound and follow discrete patterns of migration, proliferation, and differentiation across time. Combining scRNA-seq and ST can further redefine interactions between key players, such as epithelial, immune, and fibroblast cells within wounds. In additional mouse models, adaptation of complex tissue microenvironments to hypoxia during wound repair relied on secondary signals from accessory cells, such as immune cells (Konieczny et al., 2022). These findings contradict the previously held belief that epithelial cells directly sensed hypoxic conditions and subsequently responded through autonomous activation of hypoxia-inducible factor 1α (HIF1α). The authors discovered that a specific skin-resident subset of RORγt+ γδ T-cells was required for wound healing and produced IL-17A, which colocalized with HIF1α signaling transcripts at the wound front in ST analyses, suggesting an association between these immune cells and HIF1 signaling. Further functional analyses revealed that IL-17A signals to epithelial cells through the IL-17RC receptor, activating an extracellular signal–regulated kinase (ERK)1/2–protein kinase B–mTOR–HIF1α pathway. Activation of this axis ultimately directed the migration of the wound-edge epithelium through a glycolysis program (Figure 2). The implication of this glycolysis program and involvement of an IL-17A–defined axis raises new possibilities for targeted approaches in diseases driven by the same signals, such as inflammatory diseases and cancer.

The role and interaction of different cell types in promoting the disruption of wound repair processes can be further

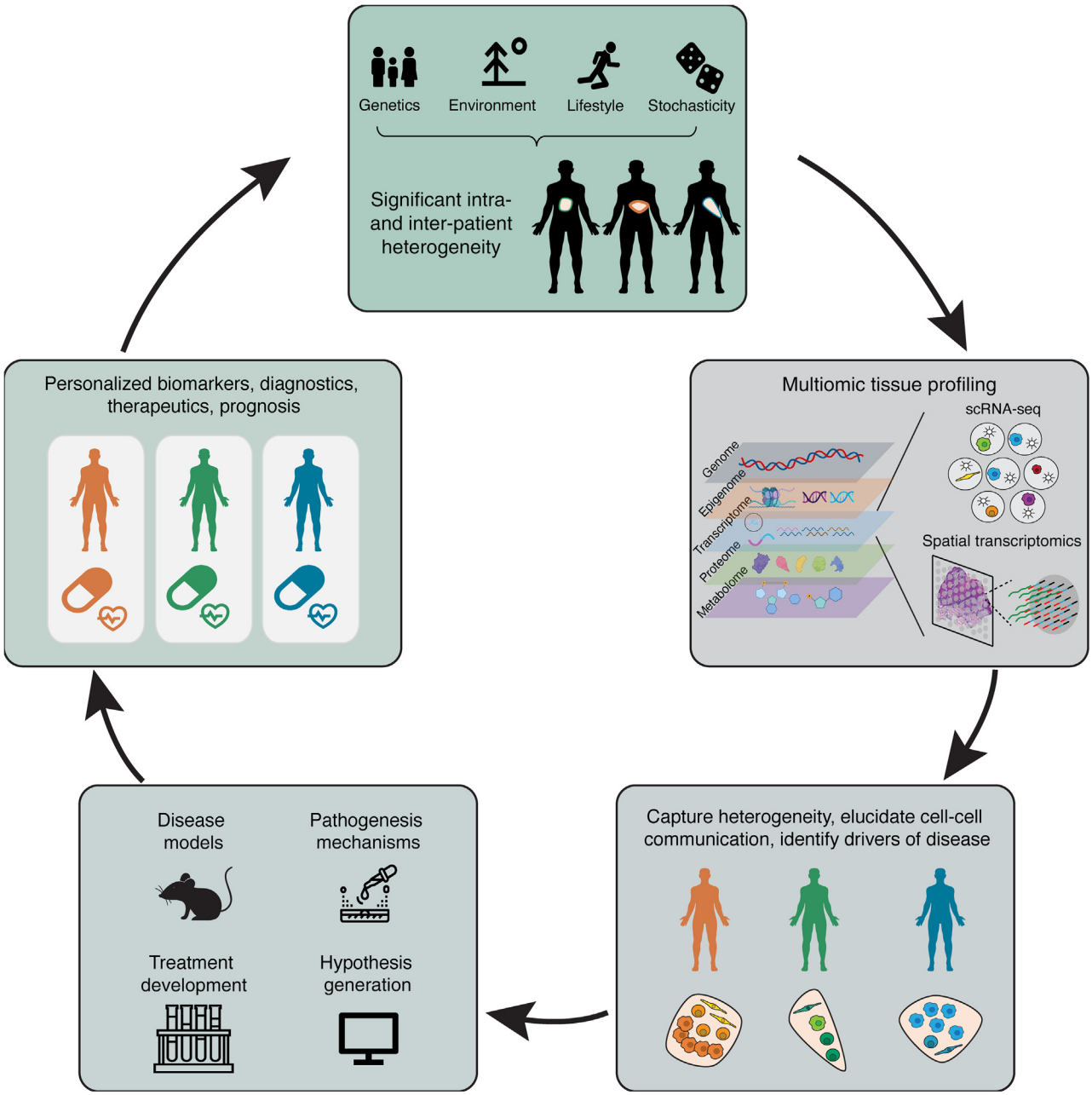

Figure 1. Idealized workflow utilizing multiomic tissue profiling for achieving personalized medicine. Workflow includes integration of scRNA-seq and ST along with other tissue profiling techniques. scRNA-seq, single-cell RNA-sequencing; ST, spatial transcriptomics.

dissected through scRNA-seq and ST. Keloids occur upon dysregulation of cutaneous wound repair and represent an overgrowth of fibrous tissue. Previously, the underlying causes for this condition have primarily focused on fibroblast contributions; however, the utility of scRNA-seq with ST has recently enabled a deeper investigation into the roles of other cell types and their interactions in driving pathogenesis. For example, single-cell profiling of human keloid and normal mature scar tissue identified populations of fibroblasts, endothelial cells, and myofibroblasts as well as disease-associated cell types and marker genes specific to keloids (Deng et al., 2021; Shim et al., 2022). Interestingly, when combining these disease-associated cell types' transcriptional profiles with their spatial information, keloid endothelial cells exhibited mesenchymal activation that could be contributing to keloid pathogenesis. In these instances, profound transcriptional changes, such as upregulation of *POSTN, TGFBR2,* and *HIF1A,* also point to the role of the surrounding low-oxygen environment in driving pathogenic fibrovascular interactions. Through ST mapping of ligand–receptor pairs and confirmation with scRNA-seq data, a dysregulated TGF-β/SMAD signaling pathway was suggested to promote neovascularization and abnormal fibrosis, further driving prokeloid mechanisms (Shim et al., 2022). These newly identifiable cellular interactions and their proposed contribution to the dysregulation of the wound healing process thus further illustrate the importance of linking expression features and locality.

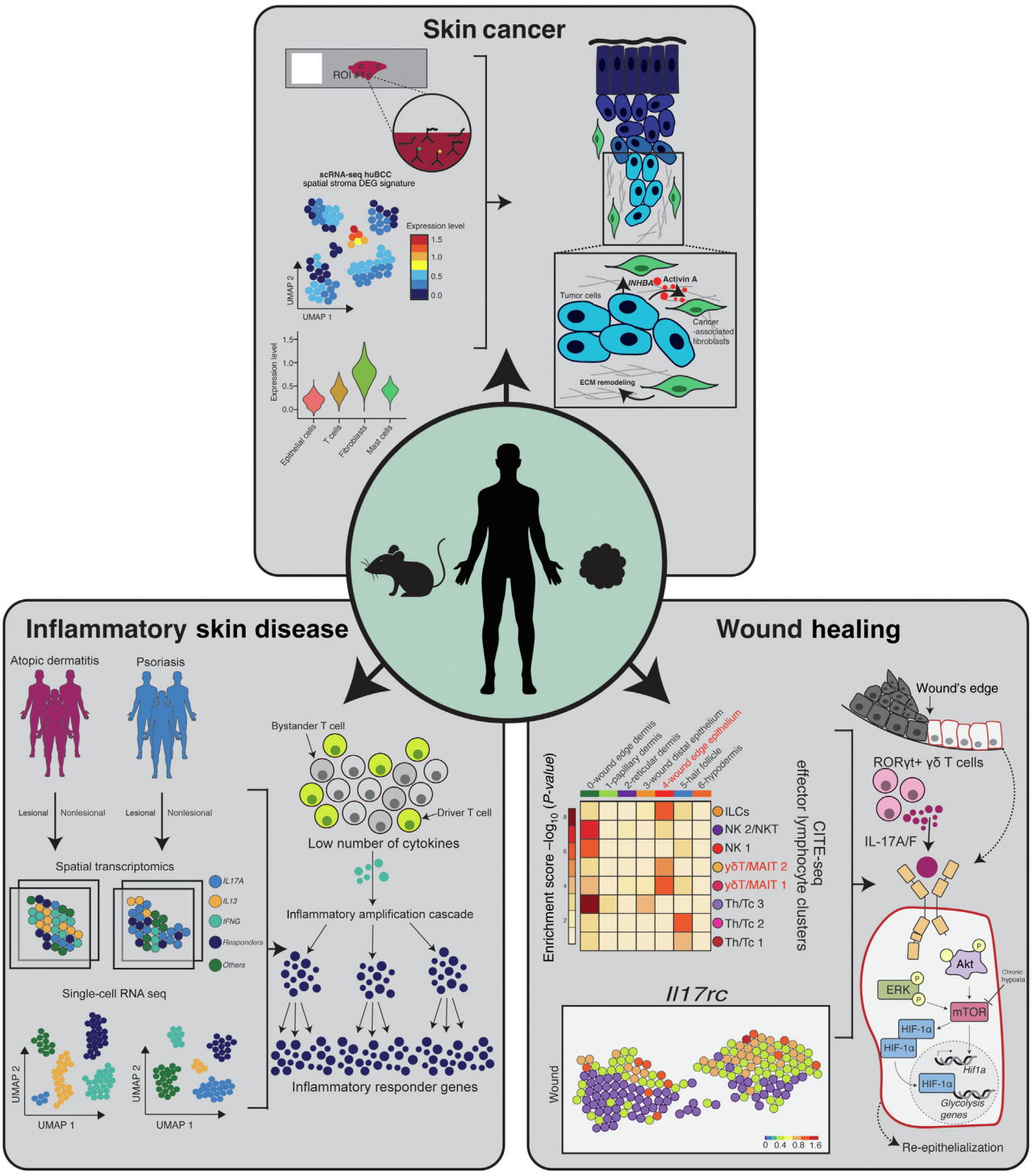

Figure 2. Examples of disease pathogenesis insights gained from combining scRNA-seq and ST. Studies have used direct profiling of patient tissue or mouse models to gain insight into cell–cell interactions involved in wound healing, inflammatory skin diseases, and cancer. Akt, protein kinase B; ECM, extracellular matrix; DEG, differentially expressed gene; ROI, region of interest; scRNA-seq, single-cell RNA-sequencing; ST, spatial transcriptomics; Th, T helper; UMAP, Uniform Manifold Approximation and Projection.

Inflammatory skin disease

Although scRNA-seq has been previously used to characterize the disease-specific subpopulations contributing to a range of inflammatory skin diseases, the combination of scRNA-seq with ST in these contexts is still in its nascency. However, a recent study employed these tools to distinguish the immune cells directly influencing disease progression from those that are purely local bystanders. The specific diseases examined were psoriasis, atopic dermatitis (AD), and lichen planus across lesional and nonlesional matched human pairs (Schäbitz et al., 2022). Interestingly, in lesional skin, the combination of only a small percentage of T cells

expressing low counts of cytokines was sufficient for initiating the inflammatory amplification cascade driving the diseases. Analyzing lesional skin across all three diseases, the spatial distribution of these cytokines was distinct, with *IL17A* enriched in all layers of the epidermis, whereas *IFNG* and *IL13* were enriched in the basal epidermis and upper dermis layers. Furthermore, driver genes, T cell genes associated with canonical cytokine expression, and responder genes induced by close interaction with cytokine-producing T cells were identified to generate gene signatures associated with T cell activation patterns across the inflammatory microenvironment (Figure 2). Ultimately, these findings illustrate the advantage of using an informed multimodal approach to identify and spatially localize disease-contributing subpopulations and their effect on the local tissue microenvironment.

Similar investigations using scRNA-seq and ST have also examined the cellular mechanisms contributing to the stages of cutaneous lupus erythematosus (CLE) immunopathogenesis. Comparison of CLE lesions with paired normal-looking skin biopsies and circulating immune cell subsets identified the existence of a primed, prelesional state in normal-appearing skin that is marked by type-I IFN-rich signaling and disrupted CCI networks (Billi et al., 2022). IFN signaling was determined to be mostly present at the interfollicular dermoepidermal junction, where both keratinocytes (KCs) and myeloid cells are localized. Upon further examination, a specific subset of myeloid cells, CD16+ dendritic cells, accumulated in both lesional and prelesional CLE environments, suggesting that these cells contribute to a lesional transition through extensive crosstalk with other implicated neighboring cells, including KCs and fibroblasts. These findings highlight the importance of deciphering cell signaling patterns and expression markers that demarcate not only the established disease state but also adjacent nonlesional tissue, which may provide clues for how inflammation progresses.

The immune system's inflammatory responses to infectious pathogen invasion also pose intriguing questions about how different initial host responses arise in the defense against foreign agents such as the leprosy-causing pathogen *Mycobacterium leprae*. In this setting, the synergism of scRNA-seq and ST provided in-depth characterization of the subpopulations of immune cells comprising the different granulomas that form and enabled interrogation of the cellular interactions contributing to antimicrobial responses across the clinical spectrum of tuberculoid leprosy (T-lep) to lepromatous leprosy (L-lep) (Ma et al., 2021). Comparison of patient samples from T-lep, L-lep, and reversal reaction (RR), where chemotherapy or spontaneous incident invokes transition from L-lep toward T-lep, found that an aggregate of mature macrophages, containing at least different two subpopulations, localized to the central zone of RR granulomas surrounded by T cells and dendritic cells in the mantle zone periphery. Furthermore, fibroblasts, KCs, and endothelial cells were implicated in the antimicrobial response in RR granulomas through the signaling of *IFNG* and *IL1B* from lymphocytes and dendritic cells, respectively. These findings contradict previous beliefs that nonimmune cell populations do not play a role in the process, thus showing the novel insights uncovered by these technologies.

Skin cancers

Cancer is a highly heterogeneous disease characterized by significant genetic and epigenetic abnormalities, further driven by complex relationships among tumor, immune, and stromal cells within the tumor microenvironment (TME). Recent advances utilizing these technologies include identifying therapeutically relevant tumor subpopulations, discerning drivers of tumor growth and invasion within the TME, and identifying biomarkers for diagnostic applications (Ahmed et al., 2022). In addition, spatial information allows researchers to better hypothesize juxtacrine and paracrine signaling networks to explicitly perturb CCIs to favorably mold the TME in the patient's favor.

Ji et al. (2020) integrated scRNA-seq and high-dimensional spatial data from both normal and cutaneous squamous cell carcinoma tissue to identify a tumor-specific KC (TSK) subpopulation located on the leading edge of tumors that commandeer tumor–stroma interactions. They identified b1-integrin ligands expressed by neighboring cancer-associated fibroblasts (CAFs), macrophages, and endothelial cells predicted to signal to TSKs that may serve as promising therapeutic targets. Scoring for the TSK signature across additional epithelial cancer types such as head and neck SCC, kidney, lung, and pancreatic cancers stratified overall survival in patients, with higher TSK scores associated with worse survival, suggesting that measuring the abundance of TSKs has the potential to predict prognosis. Yerly et al. (2022) similarly leveraged multiomics to further study the TME of the infiltrative subtype basal cell carcinoma, known to be more clinically aggressive. This effort identified a unique invasive niche consisting of tumor cells and extracellular matrix remodeling CAFs. They further identified activin A, a cytokine expressed by tumor cells at the invasive tip, as a key crosstalk molecule likely inducing known downstream targets within CAFs, such as *FN1* and *POSTN*, which were observed in scRNA-seq data and spatial expression data (Figure 2).

Pinpointing the distinct cellular alterations associated with early melanoma development is also critical to developing diagnostic strategies. Kiuru et al. (2022) leveraged ST to highlight immune and epidermal markers expressed in tight spatial clusters but lost in previous large-scale bulk RNA-sequencing studies. Of 1,400+ immunoncology-related genes measured, *S100A8*, a damage-associated molecular pattern, was revealed to be significantly expressed during melanoma growth within KCs, surprisingly, overlying melanoma in situ or malignant melanoma. Upregulation of *S100A8* was previously identified in bulk RNA-sequencing studies of melanoma, but its expression was attributed to immune cells. Immunohistochemistry (IHC) of 252 tumors validated KC-derived S100A8 expression and its calprotectin complex partner S100A9 in melanoma but not in benign nevi. Thus, cell-type–resolved ST shows the importance of probing the entire TME, with these newly identified biomarkers potentially forming the basis of additional diagnostic tools for early detection of melanomas.

THERAPEUTIC RESPONSE AND RESISTANCE

Disease heterogeneity at a patient and population level remains a crucial hurdle for achieving broad therapeutic responses in a wide range of skin diseases. For example, multiple inflammatory pathway axes can be active within a single disease, such as the coexistence of T helper (Th) 1, Th2, and Th22 cells and associated cytokines in AD. Not surprisingly, therapeutic responses directed at a single axis, such as the IL-4/-13–pathway blocking antibodies dupilumab and tralokinumab, show room for improvement, with response rates of ∼40% or less (Simpson et al., 2016; Wollenberg et al., 2021). Further complicating these outcomes is the undefined nature of cell-type–specific responses to these therapies because distinct cells within the disease microenvironment may respond differently to specific pathway blockade and potentially contribute to resistance. Thus, applying scRNA-seq and ST techniques to cohorts of responsive or nonresponsive patients to any particular therapy or in experimental models of drug resistance offer a unique opportunity to deepen our understanding of resistance mechanisms and increase response rates through improved patient selection.

Thus far, scRNA-seq has contributed more to studies of therapeutic resistance, although we anticipate that ST will provide additional details in ongoing or future work. Focusing on tumor cell–intrinsic modes of resistance, scRNA-seq has been used to better characterize minimally residual disease in *BRAF*-mutant patient-derived xenograft (PDX) melanoma samples exposed to RAF/MAPK/ERK kinase inhibition, which highlighted four distinct drug-tolerant states, including a neural crest stem cell (NCSC) subpopulation (Rambow et al., 2018). Transcriptional analyses of NCSC cells identified a reliance on retinoid X receptor signaling, the targeting of which significantly delayed relapse in PDX models. In another case study, Bangert et al. (Bangert et al., 2021) profiled the immunologic milieu in patients with AD receiving short- and long-term dupilumab treatment. Despite clinical clearance and overall normalization of KC transcriptomic dysregulation after 1 year of dupilumab treatment, scRNA-seq revealed the persistence of several populations of *LAMP3*+ dendritic, *CRTH2*+*CD161*+*IL17RB*+ Th2, and *CRTAM*+ cytotoxic T-cells, which maintained inflammatory activity through the expression of *CCL17* and *IL13*, exhibiting an inflammatory memory and potentially poised to drive disease flares. Taken together, scRNA-seq profiling before and after treatment in patients or disease models enables the identification of potential rare or unique subpopulations that drive disease relapse. Further targeting of these cells may yield more sustained responses even after cessation of treatment.

POTENTIAL FOR scRNA-SEQ OR ST AS CLINICAL TOOLS

Many of the studies mentioned earlier show that gene expression within distinct cell subpopulations identified through scRNA-seq has the potential to aid diagnosis, predict prognosis, and guide treatment. Thus, adapting scRNA-seq for clinical use could be a firm step toward achieving precision dermatology. Early efforts in characterizing rashes from indeterminate inflammatory skin conditions that are often challenging to diagnose using current clinical and histopathologic guidelines serve as an illustration of this exciting potential. Recently, scRNA-seq data on psoriasis vulgaris (PV) and AD was leveraged to uncover disease-specific cellular and transcriptomic states. Transcriptional dysregulation was observed in tissue-resident memory T (Trm) cells in both conditions, with unique and distinguishing AD-specific and PV-specific gene expression in the Trm1 subpopulation identified (Liu et al., 2022). Furthermore, rashes that exhibited higher Trm1 AD-specific gene expression were more likely to respond to dupilumab, showing the clinical significance of Trm1 gene expression. The authors further developed a clinical tool, RashX, which allows any user to input scRNA-seq data (in the form of a counts matrix) from cases of clinically indeterminate inflammatory rashes to help diagnose and match these patients with an appropriate therapeutic. Developing additional scRNA-seq–based clinical tools may hold promise for the diagnosis and treatment of other dermatologic conditions with overlapping clinical and histologic features.

Gene expression–based clinical tools are currently in clinical use for characterizing the risk of melanoma progression through the DecisionDx tool, which identifies the risk of recurrence or metastasis for patients with stages I–III melanoma on the basis of gene expression profiling (GEP) of 31 genes (Gerami et al., 2015). In Europe, the MelaGenix tool evaluates the expression of 11 genes to determine the risk of recurrence for patients with stage II melanoma (Amaral et al., 2020). GEP tests and their associated scores have been found to be clinically useful in serving as companions to determining the risk of cancer progression, highlighting the utility of transcriptomic analyses in risk stratification (Farberg et al., 2022). Still, there are limitations with GEP tests, because both tools in clinical use have been found to have limited success with correctly classifying the risk of recurrence in patients with stage I melanoma (Marchetti et al., 2020). Therefore, there is a need to gain additional insight into the transcriptomic features of various stages of melanoma, and it is possible that higher resolution assays such as scRNA-seq or ST may lead to improvement of prognosis by examining cell-type–specific gene expression, identifying rare cell types that may be masked in bulk profiling, or detecting meaningful spatial relationships among distinct cell types. However, any theoretical advantages of these approaches over current methods will need to be rigorously validated in clinical settings.

Several challenges remain for adopting scRNA-seq in the clinic. One main hurdle is the requirement of validation in large patient cohorts for diagnostic or prognostic tools, which entails cost considerations of the assay. Although the cost of sequencing has continued to decline, additional cost reductions will likely be necessary for the widespread adoption of scRNA-seq–based diagnostic tools in clinical practice. Another challenge is that scRNA-seq data analysis pipelines are currently heterogeneous and vast, with a lack of standardized guidelines for the research community (Vieth et al., 2019). We refer readers to more comprehensive recent reviews regarding scRNA-seq data analysis (Gao, 2018; Slovin et al., 2021). Variations in tissue handling protocols additionally introduce variability that limits reproducibility, given that duration and protocols may result in transcriptomic

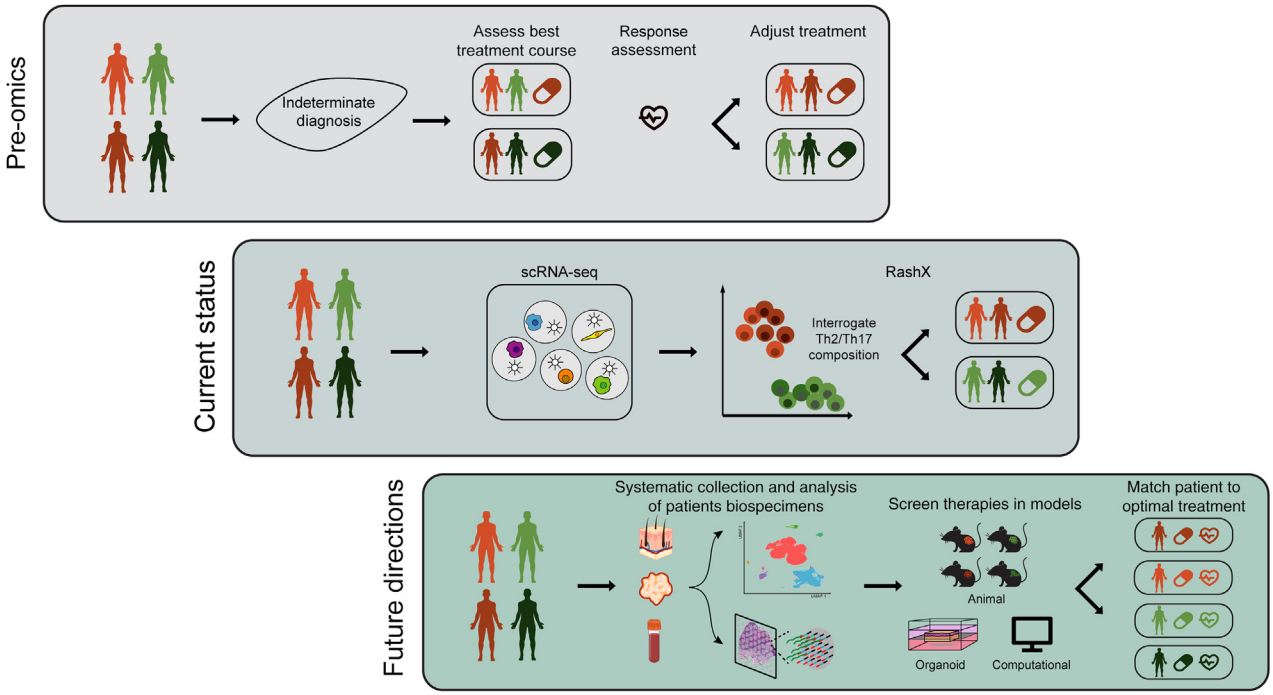

Figure 3. Comparison of workflows for matching patients to optimal treatments in three different eras. In the preomics era, patients with indeterminate diagnoses are given treatments, and adjustments are made on the basis of clinical responses to treatments. Currently, tools such as RashX enable the improvement of indeterminate diagnoses on the basis of scRNA-seq data to help match patients to the appropriate treatment strategy. In the future, we envision that leveraging multiomics will build knowledge of disease and treatment responses to eventually enable rapid assessment of biospecimens for optimizing patient treatment. scRNA-seq, single-cell RNA-sequencing; Th, T helper.

alterations (Massoni-Badosa et al., 2020). Thus, the lack of standardization in both data generation and analysis presents a challenge for the clinical use of this technology.

ST technologies offer challenges similar to those of scRNA-seq for clinical use while also bringing their own set of challenges. Namely, the wide breadth of available assays, variation in tissue compatibility and efficiency, and suitability for fresh or archived tissue are additional considerations that will need to be resolved in addition to analysis standardization. Although these challenges are not insurmountable, the technologies and analytical pipelines are far less mature than those of scRNA-seq, and more work in these areas will be required for their clinical utility to be fully realized. In summary, technologies such as scRNA-seq and ST expand the opportunities to better diagnose and treat patients in the clinic (Figure 3). However, although these technologies have proven to be powerful research tools, a potential future transition to the clinic will require more accessible and standardized workflows and a likely decrease in cost. One potential avenue for bridging the current gap in accessibility and standardization is to translate de novo scRNA-seq or ST discoveries, such as identification of one or two biomarkers, to more traditional assays such as IHC, which would be more easily validated in large cohorts. This workflow was best shown in the promising case of KC S100A8/S10089 expression in melanoma diagnosis mentioned earlier.

FUTURE DIRECTIONS

Single-cell multiomic tools have been rapidly expanding, and excitingly, their spatial counterparts are not far behind. In line with the recent development of high-throughput single-cell assay of transposase-accessible chromatin with sequencing (ATAC-seq), single-cell CUT&Tag, and single-cell whole-genome sequencing (Bartosovic et al., 2021; Casasent et al., 2018; Satpathy et al., 2019), spatial versions of each have been described in recent publications, along with high-plex imaging of epigenomic elements (Deng et al., 2022a, 2022b; Lu et al., 2022; Takei et al., 2021). Beyond genomics techniques, the field of spatial biology has grown to encompass proteomics and even functional CRISPR-based perturbation to understand the spatial biologic consequences of gene knockout (Dhainaut et al., 2022; Lewis et al., 2021). Simultaneous measurements from the same single-cell, such as joint scRNA-seq and ATAC-seq profiling, are nearly certain to encompass spatial tools in the near future (Cao et al., 2018). Thus, we are on the cusp of an exciting wave of assay development geared toward bringing together single-cell and spatial profiling technologies within intact tissue. Concurrently, the development of computational algorithms to integrate these components with additional clinical data has the potential to accelerate the transformation of knowledge into clinical care.

For therapeutic discovery to keep pace with these rapid technological advances, increasing throughput and efficiency of existing techniques are crucial for drug screening efforts of new compounds or novel combinations of therapies. Several innovations in this arena have been widely adopted into current scRNA-seq workflows. Sample barcoding or hashing through antibody- or lipid-conjugated oligos such as MULTI-seq (McGinnis et al., 2019; Stoeckius et al., 2018) has enabled pooling of samples of cells and increasing the

number of conditions or patients profiled for reaching statistical power in clinical trials, at dramatic cost savings. These techniques are indeed being applied in large-scale therapeutic testing of compounds in cancer cell lines (McFarland et al., 2020). Targeted-sequencing methods that probe a predetermined group of genes such as targeted perturb sequencing also reduce sequencing depth requirements, leading to a further reduction in costs (Schraivogel et al., 2020).

CONCLUSIONS

Currently, the combination of scRNA-seq and ST is a powerful approach for gathering high-resolution transcriptomic information in its in situ spatial context. These approaches have already shown incredible value in basic biological research, enabling the discovery of previously unclear mechanisms implicated across diseased skin states of wound healing, inflammatory disease, and cancer. Although many of these discoveries are still grounded in the research setting, the specificity of their conclusions renders inherent weight in therapeutic implications. Furthermore, as these technologies advance and become more accessible, it is likely that findings reach increasing levels of detail. From a translational perspective, the profiling potentials of scRNA-seq have already been applied in the development of accessible clinical tools, such as RashX. However, although all such work represents exciting steps forward, it is also pertinent to acknowledge the number of current obstacles faced in fully integrating these methodologies for daily clinical use. In the research environment, these challenges are largely comprised of technical hurdles and orthogonal validation; on the clinical side, there are also still many unanswered questions on how these methods would be translated directly to the clinic in a universal way. Ultimately, we believe that the continued technical improvements and standardization in scRNA-seq and ST technologies and wider adoption driven by decreases in costs will eventually lead to their integration into clinical trials, a crucial first step for supporting their use as clinical diagnostics and prognostication tools to supplement existing guidelines. In the meantime, preclinical studies showing proof of concept will be integral in the future steps toward a practical personalized medicine approach, ideally with patient cohorts defined by rigorous biological understanding and optimized and standardized protocols that maximize reproducibility. Insights gained from these studies would then inform the design of prospective clinical trials that lead to new or optimally matched treatments for patients.

Data availability statement

No original data were generated for this manuscript.

Ethics statement

No human or animal studies were conducted for this manuscript.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Supervision: ALJ; Writing — Original Draft Preparation: AEH, AK, AKN; Writing — Reviewing and Editing: ALJ

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