Single-cell transcriptomics in dermatology

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The skin is an ecosystem composed of specialized cell types that work together to serve as a physical protective barrier. Single-cell resolution is therefore essential to deconvolve skin's heterogeneity by identifying novel, distinct cell subsets in health and disease. Single-cell RNA sequencing is a highly meticulous methodology used to study the distinct transcriptional profiles of each cell within large tissue libraries at uniquely high resolution. The investigative capabilities achieved by this methodology allow previously unattainable analyses, including identification of rare cell populations, evaluation of cell-to-cell variation, and the ability to track trajectories of distinct cell lineages through development. In the past decade, application of transcriptomic analysis to skin biology and dermatology has greatly advanced understanding of homeostatic physiology in the skin, as well as a multitude of dermatologic diseases. Single-cell RNA sequencing offers tremendous promise for identification of novel therapeutic targets in dermatologic diseases, with broad implications of improving therapeutic interventions. (JAAD Int 2020;1:182-8.)

Key words: dermatology; next-generation sequencing technology; single-cell RNA sequencing; skin biology; transcriptomic analysis.

INTRODUCTION

Despite genotypic uniformity, cells have distinctive gene expression profiles reflected in their transcriptomes. Most methodologies of cellular analysis assess bulk populations and lack the specificity needed to detect such distinctions. Single-cell RNA sequencing (scRNA-seq) overcame these limitations and is a reliable, unbiased method of assessing transcriptional profiles of individual cells.[1,2] Improved operational capabilities permit high-throughput sequencing of large-scale single-cell libraries, which allows wide application of this methodology to basic science and clinical medicine.[1] The methodology of scRNA-seq and its potential applications to dermatology have been previously described by Wu et al[1]; however, rapid maturation and use of single-cell techniques have resulted in numerous dermatologic applications that have yet to be collectively examined. In this review, we will recapitulate the methodology and fundamental capabilities of scRNA-seq and summarize its previous and prospective applications to dermatology to increase awareness of its unique capabilities and catalyze its use in future investigations.

OVERVIEW OF SINGLE-CELL RNA SEQUENCING

Basics

Every cell in the human body is genetically identical; however, there is vast variability among levels of gene expression, which phenotypically manifests as cell-type specification and functional specialization. Thus, unlike the uniformity of a cell's genome, its transcriptome, or the cumulative assemblage of transcribed messenger RNA, reflects the cell's true biochemical identity. When cellular transcriptomes are examined collectively, there is clear demonstration of the intrinsic heterogeneity that exists within tissue that was once considered homogeneous. The benefits and capabilities of single-cell analyses are beyond individual cellular characterization and include the identification of *de novo* cell populations, the exhibition of intricacies of cell-to-cell variation, and the elucidation of cellular

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differentiation trajectories.[3,4] As such, the high-resolution pictures achieved by this methodology uniquely depict the distinctive roles cell subsets assume within their environment, as well as their contribution to health and disease.

Although alternative contemporary methodologies are sufficient for broad investigations, they lack the ability to finely dissect cellular involvement in molecular mechanisms, which makes comprehensive understanding of pathophysiology inaccessible. Although it may seem haphazard to put significant weight in data extracted from individual cells because available genetic material in each is scarce, the bioinformatics tools applied to such data sets can reliably detect patterns in gene expression levels that are representative of cell populations. Overall, the use of single-cell transcriptomics is rapidly advancing investigative possibilities, which offers the potential for biologic discoveries that revolutionize the understanding of disease states and thus their diagnostic and therapeutic approaches.

CAPSULE SUMMARY

- Single-cell RNA sequencing is a unique methodology that assesses transcriptional profiles of individual cells, which has greatly advanced understanding of skin biology and dermatologic disease.
- Single-cell resolution has deconvolved skin's heterogeneity and elucidated pathophysiology of health and disease, highlighting novel therapeutic targets with implications for improved clinical interventions.

Methodology

The most commonly used single-cell genomics technology is the droplet-based scRNA-seq approach, which was first described by Klein et al[5] and Macosko et al,[6] independently, in 2015. This method begins with the preparatory isolation of desired tissues, as through fluorescence-activated cell sorting. Then, with a microfluidic device, single cells are encapsulated into individual droplets, where they are labeled with a unique barcode and undergo reverse transcription to construct a single-cell transcriptome-based library. During parallel analysis of tissue libraries, the unique cellular identifiers allow retrospective identification of individual cells, which permits highly precise interpretation of findings.[5,6] Although scRNA-seq generates extensive amounts of data, typical bioinformatic analysis includes dimensional reduction and unsupervised clustering to divide thousands of cells into functionally specialized populations to visualize and interpret data[7,8] (Fig 1).

History

In 2009, the initial application of scRNA-seq was used to pedantically describe a single mouse blastomere.[9] Successive investigations quickly demonstrated the usefulness and reproducibility of this advanced methodology in immunology, cancer biology, neuroscience, and developmental biology.[10-14] Along with the robust deconvolution of tissue heterogeneity, scRNA-seq has explicated much pathophysiology of health and disease, with significant implications for improvements in clinical diagnosis and disease management. Initial investigations of skin biology and dermatologic disease using single-cell technologies have recently transpired. These foundational studies explored cutaneous cellular composition, as well as the pathophysiologic intricacies of dermatologic disease in inflammatory and autoimmune,[15-18] malignant,[19] and infectious models.[20,21]

APPLICATIONS TO DERMATOLOGY

The earliest applications of scRNA-seq to dermatology research investigated the cellular composition of normal human skin, including keratinocytes,[17] immune cells,[22] and fibroblasts[23] (Fig 2). The previously unappreciated heterogeneity of skin that was uncovered displayed precise microanatomic compartmentalization of cell subsets, which was dictated by their functional specialization. Further use of single-cell transcriptomics sought to similarly deconvolve the cellular heterogeneity of lesional skin to more thoroughly understand pathogenesis of dermatologic disease, as discussed later.

Melanoma

Transcriptional analysis provides tremendous potential for characterizing tumors and their microenvironment. Tirosh et al[19] used scRNA-seq to examine the multicellular ecosystem of genotypically variable metastatic melanoma samples. They found that malignant cells displayed abundant transcriptional heterogeneity and specifically discovered 2 distinct transcriptional states: *MITF*-high and *AXL*-high, the latter of which is associated with mitogen-activated protein kinase targeted therapy resistance. These states were previously dichotomized and bulk tumor-level classification assigned 1 to each tumor. However, with single-cell analysis, malignant cells were found to span a continuum between these

Abbreviation used:

scRNA-seq: single-cell RNA sequencing

states, which demonstrates that both tumor types contain drug-resistant tumor cell subpopulations preceding treatment. Enrichment of the *AXL*-high program occurred with targeted therapy, which biologically illustrates the clinical phenomenon of inevitable development of drug resistance. Further investigation explored the effect of tumor environment on malignant cells. The authors identified a subset of nonmalignant stromal cells whose abundance is preferentially linked to the *AXL*-high transcriptional program, as well as tumor infiltration with cytotoxic T cells.[19] These findings indicate the importance of intercellular communication for malignant phenotype, and demonstrate the potential for discovery of biomarkers capable of predicting tumor response to specific therapies.

Systemic sclerosis

Vascular injury is central to the pathogenesis of systemic sclerosis,[15] yet its mechanism is poorly elucidated. Using scRNA-seq, Apostolidis et al[16] sought to identify endothelial cell markers and signature pathways associated with vascular injury in systemic sclerosis. They found that systemic sclerosis endothelial cells expressed transcriptional profiles associated with extracellular matrix generation and epithelial-to-mesenchymal transition, and were depleted in angiogenic factors. Furthermore, they discovered that *HSPG2* and *APLNR*, genes previously associated with fibrosis, as well as vascular activation and dysfunction, are robust markers of endothelial cell injury in systemic sclerosis. These disease markers may have important diagnostic capabilities to facilitate timely intervention and thus improve disease trajectory and prognosis.

Psoriasis

Cheng et al[17] first investigated the transcriptional programming of psoriatic skin. They found that psoriatic epidermis is enriched in multiple inflammatory cell types, including channel cells, which express elevated levels of psoriasis-associated keratins; mitotic cells, which are anomalously present throughout the suprabasal layers; and a subtype of myeloid dendritic cells, which was identified as the predominant antigen-presenting cell. Additionally, they detected that inflammatory *S100* genes were elevated in keratinocytes, melanocytes, and immune cells of psoriatic skin, reflecting the multilineage response to epidermal inflammation. Further investigation of psoriatic skin using transcriptional analysis will expand on this understanding of cell-type-specific changes mediating inflammation and possibly offer new therapeutic targets.

Human papillomavirus–associated hyperplastic skin lesions

Human papillomaviruses are highly prevalent and can cause benign warts and malignancies; however, the mechanism of viral-induced epidermal hyperplasia is incompletely understood.[24] scRNA-seq was first used to study viral transcriptomes in solid tissue by Lukowski et al[20] in their effort to describe this assumed linkage between human papillomavirus infection and epidermal hyperplasia. Through comparative scRNA-seq of normal and K14E7 transgenic mice, which overexpress the HPV16 *E7* oncogene, they found that *E7* expression is predominantly enriched in basal keratinocytes and a greater proportion of *E7*-positive cells express cell-cycle- and proliferation-associated genes. Furthermore, when marker genes were compared among cell types, *E7* was one of the top genes representing basal keratinocytes.

To continue this investigation of the mechanism linking immunosuppression, human papillomavirus expression, and epidermal proliferation, Devitt et al[21] used scRNA-seq to profile human papillomavirus–positive squamoproliferative lesions. They found that a majority of cells expressed human papillomavirus transcripts, with 92% of those being identical viral subtypes. Transcriptional analysis also demonstrated upregulation of markers representative of altered skin barrier function and inflammation, including *Krt6*, which is known to modify the local immune environment and influence viral expression. This application demonstrated the usefulness of scRNA-seq in interrogation of viral expression, as well as associated pathways and molecular markers specific to human papillomavirus–induced epidermal hyperplasia.

Atopic dermatitis

He et al[18] performed the inaugural investigation of atopic dermatitis using scRNA-seq. After performing transcriptional analysis of skin from lesional atopic dermatitis, nonlesional atopic dermatitis, and healthy controls, they compiled a detailed atlas of cell populations and assessed variability in cell composition and gene expression levels between the groups. Although most cell clusters were distributed equally, the authors identified a novel

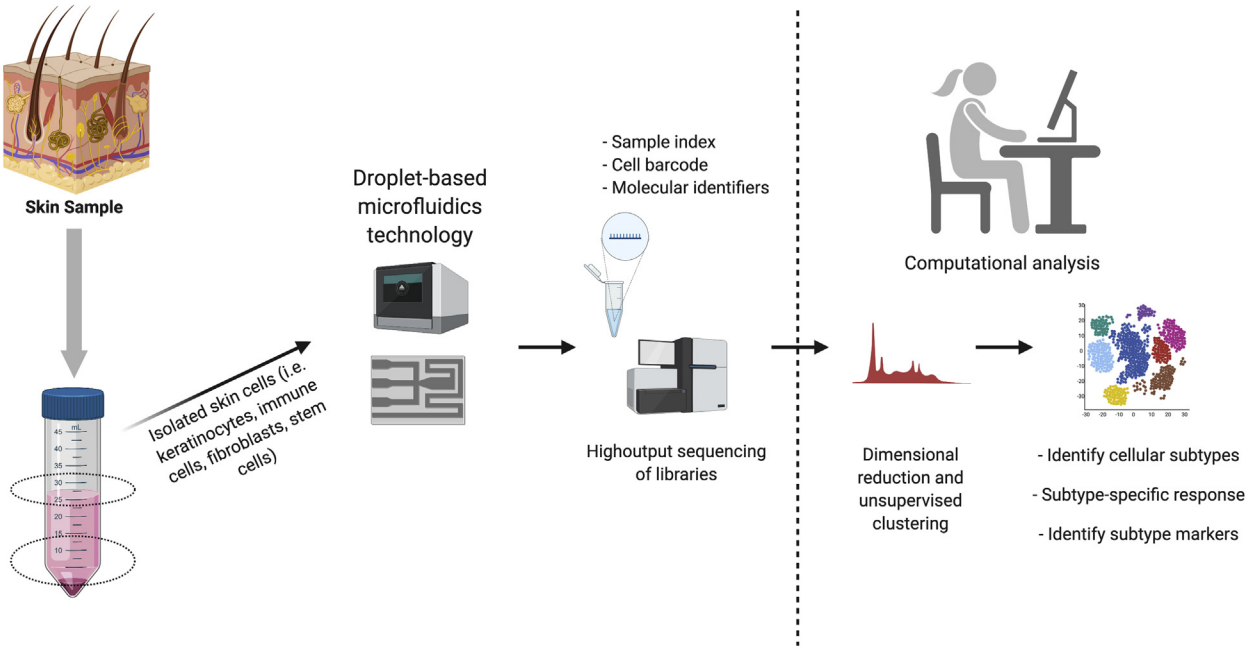

Fig 1. The work flow of single-cell RNA sequencing of skin.

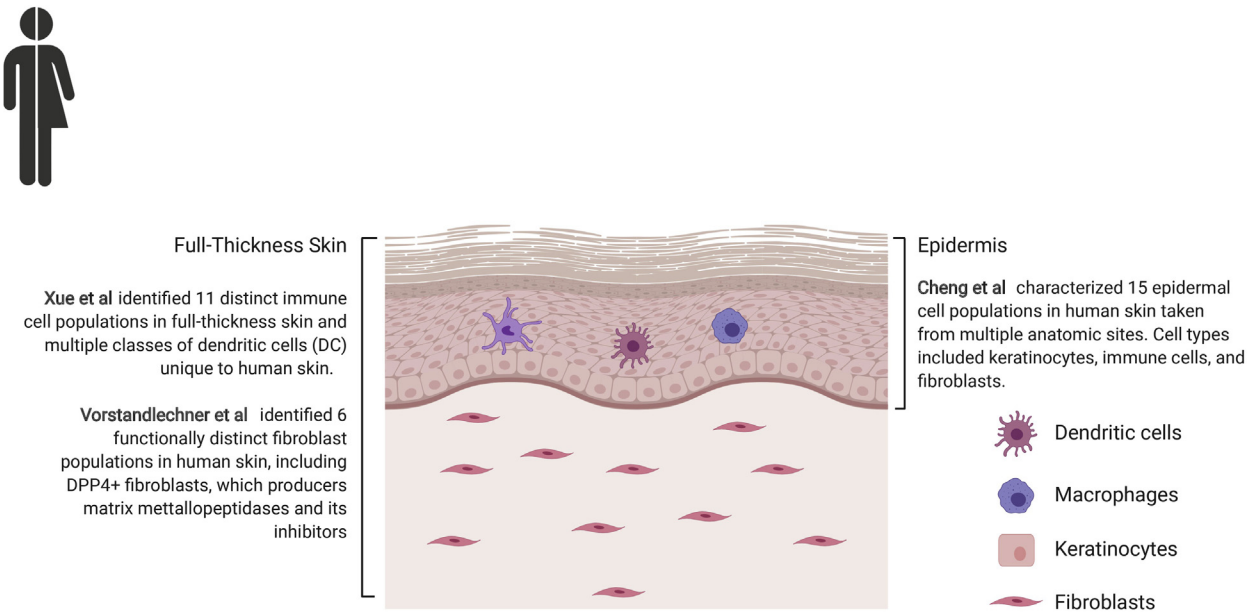

Fig 2. Transcriptome-based characterization of normal human skin composition.

subpopulation of fibroblasts unique to lesional atopic dermatitis that express proinflammatory cytokines likely responsible for lymphocyte recruitment during active disease. They also identified a corresponding dendritic cell population unique to lesional atopic dermatitis, which supports the role of intercellular communication between fibroblasts and immune cells in driving inflammation. This communication may also serve to regulate the robust type 2 inflammatory response occurring in lesional atopic dermatitis, which was found to be dampened in nonlesional atopic dermatitis and further diminished in controls. These findings contribute to the understanding of atopic dermatitis pathogenesis and demonstrate the value of transcriptional analysis on small, cryopreserved biopsies in dissecting compositional and gene expression characteristics of diseased skin.

Drug reaction with eosinophilia and systemic symptoms

Drug reaction with eosinophilia and systemic symptoms is a potentially fatal multiorgan inflammatory disease whose pathophysiology remains elusive.[25] When presented with a patient with treatment-refractory drug reaction with eosinophilia and systemic symptoms, Kim et al[26] compared transcriptomes from the affected patient with those of healthy controls and found prominent heterogeneity among lymphocytes, with drug reaction with eosinophilia and systemic symptoms lymphocytes enriched in *IL2RG*, *JAK3*, and *STAT1*. Similar transcriptional profiles were found in peripheral blood mononuclear cells, which suggests that although analysis of the primary site of inflammation is optimal, blood reflects pathology and provides an alternate specimen option. In accordance with these findings, the patient was treated with a Janus kinase-signal transducer and activator of transcription pathway inhibitor, with clinical and biochemical resolution of disease.

Cutaneous aging

As with other pathologic processes, scRNA-seq provides the unique capacity to investigate the molecular changes associated with aging. Ge et al[27] explored age-related changes in hair follicle stem cells from the back skin of young and aged mice. They found that although hair follicle stem cells' quantity declined with age, their lineage identity was maintained, with little evidence of epidermal differentiation. However, there were transcriptional changes in extracellular matrix genes, accompanied by structural alterations within the aged hair follicle stem cell niche. Marked age-related changes were also observed in nonepithelial cell types that have known influence on hair follicle stem cell behavior. Last, they discovered that although aged skin has a compromised ability to regenerate hair follicles after injury, extrinsic stimuli could override these age-related changes in hair follicle stem cells and rejuvenate their youthful behaviors. Analogously, when youthful hair follicle stem cells were introduced into an aged microenvironment, the dominance of the niche resulted in failure of their intrinsic behavior. As such, it is possible that hair follicle stem cell behavior can be functionally restored through reformation of environmental stimuli to maintain healthy aging and wound repair in aged skin.

LIMITATIONS AND FUTURE DIRECTIONS

Although incredible advancements can be credited to single-cell transcriptomics, this technology has limitations. Historically, flow cytometry has been used in skin biology to assess biochemical characteristics of cell populations, which relies on identifying cellular classification determinants to extrapolate conjectures on tissue composition and function.[28,29] However, this methodology can be applied only to cell populations with known classification determinants markers. scRNA-seq improves on this technique by allowing de novo identification of novel cell populations, although differences in experimental models and methodological variability must be taken into account when assessing the validity of findings and their interpretations. Using multimodal approaches, such as combining scRNA-seq with flow cytometry, novel cellular characteristics can be identified and systematically validated.

Another limitation of single-modal analyses is that although they fastidiously assess messenger RNA, they fail to capture epigenomic information, which is crucial for comprehensive understanding of disease pathogenesis and explains the variability in penetrance and outcomes of heritable skin disease even among monozygotic twins.[30,31] Therefore, broad application of multimodal technology will be the preeminent investigative approach for study of skin biology. These approaches could be further optimized by increasing throughput capabilities to streamline analysis of large tissue libraries; for example, the skin composes approximately 16% of total body weight, accounting for about 1.6 trillion cells. Current single-cell technology can analyze libraries up to 100,000 cells,[32] which is a negligible representation of skin as a whole. Thus, increasing throughput will achieve a more comprehensive and unbiased picture.

Despite these limitations, continued application of the persistently advancing technologies of single-cell transcriptomics to skin biology will achieve a more comprehensive understanding of dermatologic disease. Although investigative possibilities are numerous, single-cell approaches will be central in exploring dermatologic diseases whose pathogenic mechanisms remain unexplained by alternative inspective methodologies. For instance, ongoing investigations of multiple inflammatory diseases, including atopic dermatitis and hidradenitis suppurativa, are attempting to dissect the relationship between cutaneous inflammation and microbial dysbiosis.[33,34] Determining temporality and causality among these pathologic components through pairing single-cell transcriptomics with bacterial analyses will provide new insight into disease pathogenesis and offer an innovative perspective on therapeutic approaches. Additionally, single-cell analyses will allow thorough characterization of the malignant cellular components of various skin cancers, which

can provide valuable information for the relative efficacy of targeted therapies or immunotherapy in individual patients, as well as provide a foundation for customization of novel therapeutics. These advancements support the pioneering model of precision medicine, which offers individually tailored treatment options for patients with disease that is otherwise difficult to treat.

CONCLUSION

The unique capabilities of scRNA-seq have greatly advanced understanding of skin biology, as well as pathophysiology of multiple dermatologic diseases. From these foundational studies, a detailed atlas of cell populations in healthy and diseased skin has been created and cell-specific gene expression analyzed. Although cost and absence of a data analysis tool with equivalent precision remain prohibitive, persistent improvement in next-generation sequencing technologies encourages their continued application. The wide array of potential applications for this methodology offers substantial promise for identification of novel therapeutic targets in numerous dermatologic diseases, with the attainable long-term goal of improving management.

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