MEDICAL SCIENCE MONITOR

REVIEW ARTICLES

e-ISSN 1643-3750
© Med Sci Monit, 2021; 27: e931862
DOI: 10.12659/MSM.931862

Received: 2021.02.28
Accepted: 2021.05.06
Available online: 2021.05.14
Published: 2021.05.20

Applications of Single-Cell Sequencing in Dermatology

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: This work was supported by Yunnan Province Clinical Research Center for Skin Immune Diseases [grant number 2019ZF012]; Yunnan Science and Technology Leading Talents Project, Young and middle-aged academic and technical leaders reserve talents [grant number 2017HA010, 2017HB044]; and PhD Innovation Fund Project of Kunming Medical University [grant number 2020D003]

Single-cell sequencing (SCS) is a promising new technique used to assess the genomics, transcriptomics, epigenetics, and other multi-omics at the single-cell level. In addition to elucidating the immune microenvironment and revealing the pathomechanisms of disease and drug resistance, SCS can profile the actual state of an individual cell and identify a novel cell type and differentiation trajectories, which cannot be achieved by bulk tissue sequencing technique. SCS technique serves as powerful tools to explore more meaningful biomarkers of diagnosis, prognosis, and new therapeutic targets in clinical practice. The SCS technique has been widely applied in the field of dermatology. In this review, we summarize the advances of SCS in dermatology.

Keywords: Cellular Microenvironment • Dermatology • Gene Expression Regulation • Genetic Heterogeneity • Molecular Targeted Therapy • Single-Cell Analysis

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/931862

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Background

Single-cell sequencing (SCS) is a new technology for high-throughput sequencing analysis of genome, transcriptome, and epigenome at the level of a single cell. Almost all tissues and organs are composed of various types of cells. Compared to bulk sequencing of multicellular samples, SCS can determine the expression pattern of a precise gene in thousands of single cells, and comprehend the function and gene expression status of individual cells and the interaction between cells. In addition, SCS can identify new/rare cell populations, explore their function, and solve the problem of cellular heterogeneity, which could not be achieved by bulk sequencing. Since the first application of high-throughput single-cell whole-transcriptome sequencing in 2009 by Tang et al [1], the SCS technique is becoming a powerful tool for making breakthroughs in life science and clinical medical research. Now the SCS technique has been employed in single-cell genome sequencing (scDNA-seq), single-cell transcriptome sequencing (scRNA-seq), single-cell DNA methylation sequencing, single-cell histone modification sequencing, and single-cell chromatin structure sequencing (scATAC-seq). The main workflow includes: single-cell suspension preparation, single-cell capture and labeling, library preparation and sequencing, and data analysis. The commonly used platforms are Smart-Seq2, 10x Genomics, and Fluidigm C1.

Skin is the largest organ of the human body, with a complex structure and multiple cell types, exerting multiple functions such as permeability barrier, immunity, antimicrobial defense, and metabolism. The interactions between cells maintain cutaneous homeostasis and involve in the pathogenesis of diseases. Now SCS technique has been widely used in the analysis of both normal skin and diseased skin to understand the cutaneous biology, wound healing, pathogenesis, and progression of skin disorders. Here, we comprehensively review the progress of SCS applications in the field of dermatology.

Normal Skin And Hair Follicles

Normal Skin

It is generally assumed that the same type of cells exerts the same function. However, results of SCS defy our conventional wisdom. Using scRNA-seq to analyze the transcriptional characteristics of keratinocytes (KCs), Cheng et al [2] showed that the KC subpopulations and inflammatory genes expression differed in the scalp, trunk, and foreskin. In particular, inflammation-related markers such as *S100A7, S1008, S100A9,* and *IFI27* are upregulated in normal scalp interfollicular KCs, likely due to inflammation and pruritus in the scale, while in the foreskin, levels of proliferation-related transcripts are upregulated accompanied by increased *IL1β*^{hi}*CCL3*^{hi}*CD14*$^{+}$ macrophages, which may play a role in immune surveillance.

In the traditional view, dermal fibroblasts are classified into papillary and reticular fibroblasts. However, analyses with SCS technique revealed that dermal fibroblasts consist of at least 4-7 different subpopulations in skin samples from the dorsal forearm, the female breast, and the abdomen [3-5]. Utilizations of spatial transcriptomics and RNAScope in situ hybridization show variation in spatial distribution of fibroblast subpopulations, suggesting that different fibroblast subpopulations may play distinct roles in extracellular matrix (ECM) production, inflammation, and epidermal remodeling. Recent studies demonstrated that the potential interactions of aged fibroblasts with other cells were significantly reduced in comparison to young fibroblasts [6]. Moreover, 3 macrophage and 6 dendritic cell (DCs) subpopulations, with distinct gene expression profiles, were identified in healthy human skin by scRNA-seq [7]. Identification of subpopulations of cells can improve our understanding of cutaneous biology.

Hair Follicles

Hair follicles (HFs) are complex skin appendages with strong self-renewal and regenerative capacity, and their evolution and regeneration involve many cell populations. The outer root sheath (ORS) is composed of 2 transcriptionally distinct cell types with different abilities to interact with stromal cells, while the inner root sheath (IRS) is generated from transcriptionally uncommitted progenitor cells and coordinate hair production with ORS. Although bulk sequencing can assess expression levels of genes, it cannot differentiate cell types. In contrast, the scRNA-seq technique can identify 25 different cell populations, including previously unknown cells, based on cell marker genes [8]. In addition, using scRNA-seq and single-molecule mRNA fluorescence in situ hybridization (smFISH), Joost et al mapped the gene expression characteristics and spatial positioning of full-thickness mouse skin during hair growth and rest, and identified 56 epithelial and stromal cell populations [9]. Moreover, Wang et al [10] showed that Oncostatin M (an endogenous inhibitor) was mainly produced by TREM2+ macrophages in the early telogen phase, which maintain hair follicle stem cells (HFSCs) quiescence by negative regulation of the hair growth process induced by the JAK-STAT pathway, while TREM2+ macrophages decreased after the telogen phase. These results indicate that scRNA-seq is useful to identify different cell subpopulations in hair follicles.

Recently, Ge et al [11] employed scRNA-seq to elucidate the dermal role in the development of hair follicles. They found that although the aged HFSCs were reduced in numbers and activity, they maintained their HFSC identity within the bulge. Young HFSCs, but not aged HFSCs, regenerate HFs when the

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skin is injured. However, aged HFSCs can regenerate HFs when supported with young dermis, while young HFSCs fail to regenerate HFs when combined with aged dermis. This study points to the importance of the stem cell niche as a key modulator of stem cell behavior.

Wound Healing

Cutaneous wound healing is a complex process. Basal cells are essential for maintaining the integrity of skin and promoting wound healing. Haensel et al [12] used scRNA-seq combined with RNA in situ detection (RNAScope) and fluorescence lifetime imaging microscopy (FLIM) to dynamically monitor cell states during skin homeostasis and wound healing. In their study, 3 non-proliferative states and 1 proliferative state with different metabolic profiles were identified in the basal layer during homeostasis, and were spatially separated in wound epithelialization. In wound healing, the inflammatory genes *Cxcl2, Ccl2,* and *Ccl7* and epithelial-to-mesenchymal transition (EMT)-related genes *Snai2* and *Vim* are upregulated in basal cells. Guerrero-Juarez et al [13] revealed the wound fibroblast diversity and myeloid-derived adipocyte progenitors by scRNA-seq analysis of cells from mouse wound dermis 12 days after wounding. They distinguished 3 distinct fibroblast-to-myofibroblast differentiation trajectories and 12 fibroblast subpopulations, and predicted fibroblasts differentiation trajectories via pseudo-time and RNA velocity analysis. In addition, their studies showed that differentiation of myeloid-originated myofibroblasts to adipocytes benefits wound repair. Other studies also demonstrated that generation of diverse ECM-producing myofibroblasts within wounds by adipocyte-derived cells contributes to skin wound healing [14]. Moreover, scRNA-seq technique reveals that skin adipocyte lipolysis plays a key role in regulating inflammation of efficient macrophage after skin injury in genetic mouse models. Furthermore, Guerrero-Juarez et al [15] used scRNA-seq to analyze the healing ability of fibroblasts from young and old mice, and found that old mice with slow healing had more fibroblasts, whereas fast-healing old mice had more immune cells in the wounded site. This result highlights the possibility of personalized treatment of cutaneous wounds in the future.

Inflammatory and Allergic Skin Diseases

Psoriasis

Psoriasis, one of the top 10 recalcitrant chronic diseases listed by the WHO, is an immune-mediated chronic inflammatory skin disease with unclear pathogenesis. Over the last decade, scRNA-seq has advanced the progress of understanding of the pathogenesis of psoriasis. Cheng et al [2] used scRNA-seq to analyze the heterogeneity of KCs in different parts of 9 normal and 3 psoriatic skin samples. They found more proliferation-related and cell signaling-related KC subpopulations in psoriasis skin lesions. The inflammatory transcription level of *S100, IFI27,* and *PI3* and cell division are significantly enhanced both in basal, spinous, and granular KCs. More *CD1C*$^+$*CD301A*$^+$ myeloid DCs are enriched, while fewer macrophages and Langerhans cells (LCs) are enriched in the epidermis, suggesting that the proliferation and recruitment of DCs further promote the activation of effector lymphoid and myeloid cells to provoke the development of psoriasis.

In addition, a large body of evidence indicates the association of psoriasis with abnormal metabolism. Lou et al [16] analyzed psoriatic epidermis with scRNA-seq and revealed high expression levels of the arginine biosynthesis rate-limiting enzyme, Arginosuccinate synthetase1 (ASS1), in basal KCs, suggesting that basal KCs are the source of arginine in the skin and contribute to epidermal hyperproliferation. Correspondingly, arginase inhibitors improve hyperplasia and inflammation in the epidermis of mouse psoriasis-like models.

Atopic Dermatitis

Atopic dermatitis (AD) is also a very common inflammatory skin disease. He et al [17] performed scRNA-seq on 39 042 single-cell skin samples from 5 AD patients and 7 healthy controls to construct a comprehensive single-cell transcriptome map of all cell types from healthy skin and from AD lesional and non-lesional skin. The results revealed the changes in composition and specific gene expression, and identified a unique fibroblast subpopulation, *COL6A5*$^+$*COL18A1*$^+$ fibroblasts, in AD lesional skin. *COL6A5* is an AD susceptibility gene, which can cause AD through the formation of unstable heterotrimers, resulting in abnormal fibroblast adhesion, collagen synthesis and metabolism, and barrier disruption, while *COL18A1*/endostatin can promote allergen sensitization and inflammation by regulating LCs and T cells.

Drug Eruption

Drug-induced hypersensitivity syndrome (DiHS) is a fatal systemic drug reaction with a high mortality rate, which is characterized by acute extensive skin lesions, multiple-organ damage, eosinophilia, and human herpesviruses (HHV6b) reactivation. Kim et al [18] performed scRNA-seq, single-cell T cell receptor sequencing (scTCR-seq) on skin and blood samples from a patient with DIHS (induced by sulfamethyisoxazole/trimethoprim and uncontrolled by high doses of hormones) and 5 healthy volunteers. They sequenced 4676 single cells from DiHS skin and 13 542 cells from healthy volunteer skin. A total of 14 cell clusters were clustered by Unsupervised t-SNE. Extensive transcriptomic alterations were found in immune and nonimmune

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cell types, particularly in lymphocyte clusters. The pathway enrichment analysis with differently expressed genes (DEGs) showed that the JAK-STAT signaling pathway was most active, and the DNA from HHV6b was found to be highly enriched in $CD4^+$ T_{CM}. TCR chain sequencing by scTCR-seq showed significant differences in T cells, high expression of chemokine receptors in lymphocytes, and upregulation of genes related to the JAK-STAT signaling pathway in the patient with drug hypersensitivity reaction syndrome. Based on the results, tofacitinib (an inhibitor of JAK1 and JAK3) was given to the patient. In addition to the improvements in clinical condition, treatment with tofacitinib reduced $CCR4^+CCR10^+CD4^+$ and $CD8^+$ T cells as assessed by scRNA-seq. Finally, studies using a lymphocyte transformation test (LTT) to simulate drug-induced immune responses in vitro confirmed that tofatinib and antiviral drugs inhibited $CD4^+$ T cell proliferation and downregulated JAK-STAT pathway-related genes. These results suggest the usefulness of scRNA-seq in identification of potential therapeutic target and the therapeutic utility of JAK inhibitors and antivirals for DiHS.

Autoimmune Dermatosis

Vitiligo

Vitiligo is an autoimmune skin disease that seriously affects the appearance of patients. Studies have concluded that vitiligo is not solely related to melanocytes, but is the result of a combination of cell populations across the entire layer of the skin. Lin et al [19] performed scRNA-seq to analyze 11 000 cells of skin samples from 2 patients with stable non-segmental vitiligo and 2 healthy controls. They found that the stem cell subpopulation in vitiligo lesions was significantly lower than that in normal skin, while the fibroblast subpopulation was absent or insufficient in number, and differentiated into myofibroblasts with high expression of α-smooth muscle actin, along with excessive accumulation of intracellular reactive oxygen species. In addition, the volume of fibroblasts and adhesion proteins increased, exhibiting an aging phenotype, leading to dysfunctions in skin nourishment and repair. Although the general proportion of KCs did not differ, 4 subpopulations of KCs at the end of cell differentiation were significantly higher in vitiligo skin than in normal skin. KC subpopulation 5 highly expresses *KRT15, POSTN, COL17,* and *S100A2* genes, which are related to intercellular connection, cell adhesion, and cytoskeleton function; KC subpopulation 6 highly expresses cellular differentiation, oxidative stress-related genes, *SFRP1, GJB* family, *SOX9*, and *KRT6*; KC subpopulation 10 highly expresses cell homeostasis and cell cycle-related genes *PTTG1*, *CENPF*, *HMGB2*, *TOP2A*, *UBE2C*. The characteristics of these cell subpopulations suggest that they are associated with vitiligo occurrence and progression.

To investigate the signaling networks that contribute to the pathogenesis of vitiligo, Strassner et al [20] used a modified suction blistering technique to isolate skin cells directly from 6 vitiligo subjects with active disease, and analyzed more than 18 000 multiple cell populations, including melanocytes, KCs, and immune cells. Their results showed an IFN-γ signature, increased $CD8^+$ T cell, and 87 ligand-receptor pairs, including CXCR6 and CXCL16, which can promote vitiligo progression. Importantly, LCs were found to express many genes that are associated with vitiligo, suggesting that LCs play an essential role in the development of vitiligo.

Alopecia Areata

Alopecia areata (AA) is a disorder of T lymphocyte-mediated autoimmune hair loss. The "normal appearance" of smooth and non-inflammatory skin in the affected area contrasts sharply with the complex immune activity occurring at the HFs. The loss of immune privilege of HFs resulting in an autoimmune attack contributes to the development of AA. Borcherding et al [21] applied scRNA-seq and scTCR-seq to study 18 231 immune cells from the skin and lymph nodes of the C3H/HeJ AA mouse model and control group to explore the role of the complex skin immune ecosystem, microenvironment, and other cells in the pathogenesis of AA. They found that $CD11b^+$ $CCR2^+$ myeloid DCs (mDCs) enriched the JAK/STAT signal, and the expression of MHC-I and MHC-II genes in APCs increased, indicating an increased immune reactivity and loss of immune privilege in AA. Moreover, $CD4^+$ and $CD8^+$ T cells in AA were clonotypically expanded, and TCR sequences were extensively shared between different cell clusters in AA, but not in the control group, suggesting an antigen-specific immune response in AA. Results in the animal model were validated by scRNA-seq on 2416 T cells from human AA lesions. Based on differentially upregulated genes in $CD4^+$ and $CD8^+$ T cells in a murine AA model, the authors used a 15-gene signature (eg, *TAP1, PSMB9*) to predict human AA. The results showed that $CD4^+$ and $CD8^+$ T cell gene signatures had equivalent performance, with an overall accuracy of 87.5%, sensitivity of 90%, and a specificity of 83.3%.

Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a common, intractable autoimmune disease that can extensively affect the skin, kidneys, joints, blood system, and central nervous system. Lupus nephritis (LN) is a leading cause of mortality in SLE patients. Der et al [22] implemented scRNA-seq on 5000 cells from skin samples from healthy controls, nonlesional, and non-sun-exposed skin of LN patients, and 15 000 kidney cells. Interestingly, they found upregulation of the type I IFN response pathway in KCs and the kidney of LN patients. Goel et al [23] demonstrated that immune cells were not extensively responsive to IFN-λ, but the KCs and mesangial cells responded to IFN-λ, and

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produced chemokines to induce immune cell recruitment and to promote systemic immune dysfunction in SLE. Because IFN-λ and type I IFN share some functional similarities, new treatment measures for the shared elements of type I and type III IFN may be effective for SLE patients.

Scleroderma

Systemic scleroderma (SSc) is a chronic connective tissue disease with extensive hardening of the skin and connective tissue. It is generally believed that the pathogenesis is mainly the abnormal activation of fibroblasts, leading to scarring of skin and internal organs. However, the first event in most SSc patients is microvascular injury, not fibrosis, suggesting that vascular endothelial injury may contribute to the pathogenesis of SSc. Apostolidis et al [24] used scRNA-seq to analyze the endothelial cells of SSc skin lesions and healthy skin. They found that the endothelial cells of SSc highly expressed *VWF, THBS1, APLNR,* and *HSPG2*, which are mainly related to vascular injury and activation, ECM generation, and negative regulation of inhibiting angiogenesis. Both *APLNR* and *HSPG2* are 2 main creators of SSc endothelial cell injury as assessed by IHC, immunofluorescence, and qPCR.

Infections

HSV-1

Herpes simplex virus-1 (HSV-1) is one of the viruses with the highest infection rate in the world, affecting 45%-88% of the global population [25]. Although 80% of the viruses are static latent infection, they can replicate in large numbers and cause various diseases after activation. Wyler et al [26] utilized scRNA-seq to accurately detect about 12 000 primary normal human dermal fibroblasts (NHDFs) infected with HSV-1 in vitro at different early stages (1, 3, and 5 h after infection). They also showed that the main cause of cell heterogeneity is cell cycle and infection processes. Cells in the S/G2/M phases are more susceptible to viral infection, and the virus replicates faster in these cells. In addition, expression of *NRF2* is positively correlated with viral transcripts through RNA velocity analysis, while *NRF2* can slow down the process of infection activation. Thus, activation level of *NRF2* can serve as a marker of resistance to HSV-1 infection. Bardoxolone methyl and Sulforaphane, 2 known *NRF2* agonists, can reduce the mRNA expression of the early virus gene *UL29* and late virus gene *UL6*, indicating the decline of the virus's ability to replicate. These results suggest that scRNA-seq is useful to detect early HSV-1 infection and to identify therapeutic targets.

The combination of scRNA-seq and live-cell imaging techniques revealed that during HSV-1 infection, only a few cells become highly infected, largely due to shutting down the signal transduction process that protects against the virus [27]. Authors also showed that viral gene expression level in infected cells at G2 phase is very low. Viral gene expression is negatively correlated with cell cycle score. Interestingly, highly infected cells undergo cell reprogramming, which involves the recruitment of β-catenin into the host nucleus and virus replication regions required for late viral gene expression and progeny production. Other studies using SCS demonstrated that lncRNA plays an important role in host cells infected by HSV. Silencing lncRNA MAMDC2-AS1 can reduce HSV-1 immediate early (IE) genes expression in human host cells to limit HSV-1 infection [28].

HPV

Human papillomavirus (HPV) infection is also highly prevalent worldwide; it can induce warts and cancers. HPV-related tumors are highly correlated with HPV16. Lukowski et al [29] used SCS on KCs from K14E7 transgenic mice, a precancerous hyperproliferative epithelium model, to explore the relationship between HPV16 infection and cutaneous squamous cell carcinoma (cSCC). They observed that the expression of E7 is mainly associated with basal KCs, while E7 can upregulate multiple cell cycle- and proliferation-related genes. The increased E7 transcript copy number is positively correlated with the expression level of E7-induced genes.

Persistent HPV infection of skin can occur in patients with chronic immunosuppression such as after organ transplantation. These patients usually have more severe symptoms and are more likely to develop tumors. Devitt et al [30] implemented HPV genotyping and scRNA-seq to analyze 3 wart lesion samples and normal skin of an immunosuppressed patient with HPV infection, and found that the HPV transcripts of different lesions in the same patient are heterogeneous. Although more than 92% of cells in each lesion express HPV78, the markers associated with skin barrier function (*ARL4A*, *MT2A*) and inflammation (*FOS* and *JUN*) were upregulated in 2 out of 3 lesions. Moreover, the expression levels of stress keratins such as *KRT6A* and *MT1G* genes were upregulated in 1 lesion. Previously, Zhussupbekova et al [31] showed that *KRT6A* could modify the local immune environment and alter the virus expression, and *MT1G* was upregulated in precancerous lesions with HPV16 infection. In addition, the proportion of cells in G1 phase was higher, and that in S phase was lower in 1 lesion, suggesting these cells may be precancerous cells.

SCS technique has also been used to study human cytomegalovirus (HMCV) [32] and human immunodeficiency virus (HIV) [33]. These studies are of potential significance for further exploration of the interaction between virus infection and host in the pathogenesis of viral infections, as well as for the development of virus vaccines and immunotherapy.

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Bacteria

Skin-resident immune cells play an important role in antimicrobial defense. Conventional dendritic cells (cDCs), including cDC1s and cDC2s, are the main antigen-presenting cells (APCs), with dual functions of activating immune response and immune tolerance. Janela et al [34] used single-cell mRNA sequencing to reveal the interactions between skin APC subsets and other immune cells during *Propionibacterium acnes* (*P. acnes*) infection. They found that EpCAM$^+$CD59$^+$ ly-6D$^+$cDC1s, a subpopulation of cDC1s, can secrete VEGF-α to recruit neutrophils to the inflammatory site and to regulate the immune response induced by *P. acnes* in the dermis of mice. Thus, SCS technique can differentiate the roles of different cDCs subsets in regulating innate immunity.

Innate lymphoid cells (ILCs), which are tissue-resident cells, exert antimicrobial properties. The mechanisms of ILC action are not clear yet. Studies using scRNA-seq demonstrated that ILCs in different sites are highly heterogeneous in the skin [35]. Persistence of ILCs in the skin relies on IL-7 and thymic stromal lymphopoietin, while its localization is determined by cytokines secreted by epithelial cells and chemokine receptor CCR6. Interactions of ILCs and HFs and sebaceous glands regulate host-microbe symbiosis. Deficiency of ILCs results in sebaceous hyperplasia with an increase in antibacterial lipids to inhibit the symbiosis of gram-positive bacteria.

Skin Tumors

Tumors cells lose their normal growth control and proliferate abnormally. To adapt to the complex tumor microenvironment (TME) in the host, the tumor cells change constantly, resulting in many gene mutations and exhibition of unique pathogenic phenotypes, termed intratumoral heterogeneity. The development of scRNA-seq has provided a powerful tool for exploring the heterogeneity, occurrence, and development of tumors, the mechanism of drug resistance, and new treatment strategies.

Melanoma

Melanoma is a type of skin malignant tumor with high rates of invasion, metastasis, and recurrence, and the highest mortality rate among skin cancers. In recent years, both targeted therapy and immunotherapy have helped melanoma patients, but because of the high heterogeneity of melanoma, the outcome is still not satisfactory, and more effective specific therapeutic targets are needed. Tirosh et al [36] analyzed the heterogeneity of tumor cells and their interactions with TME in 19 cases of melanoma using scRNA-seq. Two groups of tumor cells – high MITF subpopulations and low MITF/high AXL subpopulations – were identified. Importantly, high AXL tumor cell subpopulations were associated with resistance to RAF and MEK inhibition. Tumors with more CAF enrichment expressed the AXL-high signature, while the MITF-high tumors were negatively correlated with CAF abundance. In addition, expression of key T cell co-inhibitory receptors such as PD-1, TIM-3, and CTLA-4 suggests that T cells in melanoma present a state of exhaustion and may be used as targets for immunotherapy. Su et al [37] utilized single-cell proteomics and metabolic analysis on BRAFV600E mutant melanoma cancer cell lines cultured for 0, 1, 3, and 5 days following stimulation of melanoma cells from drug-responsive state to drug-tolerant state. The results showed that the key transcription factor MITF, metabolism regulators HIF1 and P-AMPK, proliferation marker Ki67, and glucose metabolism changed significantly at different time points. Three-dimensionality reduction algorithms (FLOW-MAP, t-SNE, and PHATE) were used to visualize the changes in multiple substances at the single-cell level at different time points after stimulation. Two distinct paths and MITF were identified as key molecular drivers for the bifurcation of the trajectory. This single-cell multi-omics study has greatly deepened our understanding of the transformation of tumor cells resistance, leading to the development of new therapies for melanoma.

Recently, Davidson et al [38] used scRNA-seq to investigate changes in TME and lymph node status during the development of melanoma in mice. They found that T cells recruited from the lymph nodes were immediately activated and expanded, in addition to exhibition of exhaustion state in the late stage of the tumor. They also identified 3 distinct functional populations of tumor stroma (S1-S3) with different immunity, proliferation, and contractility functions. S1 and S2 cells predominated in the early stage, and S3 populations were largely restricted in the late stage. Cell-cell communication analysis showed that complement Component C3 was specifically expressed in the immune population, and its cleavage product C3a played an important role in regulating immune populations. The combination of C3a and C3aR interferes immune infiltration and inhibits tumor growth, suggesting that stromal cells are important in regulating disease immune function.

Cutaneous T Cell Lymphomas CTCL

Cutaneous T cell lymphomas (CTCLs), including mycosis fungoides (MF), Sézary syndrome (SS), and other types, are T cell lymphomas with skin damage as the main manifestation. Although most CTCLs lesions are limited to the skin, advanced-stage CTCLs can also involve system organs and cause death. So far, treatment regimens are limited. Gaydosik et al [39] analyzed 14 056 CD3$^+$ T lymphocytes from patients with advanced-stage CTCL and healthy human skin by scRNA-seq. They observed that the cellular profiles were similar among individual normal skin samples, whereas CTCL samples were highly heterogeneous, especially in lymphocytes, and that different lymphocyte populations activated specific tumor-related pathways. High expression levels of PCNA, ATP5C1, and NUSPA1 in lymphocytes can be used as biomarkers for the diagnosis of

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CTCL. In advanced CTCLs, multiple co-inhibitor receptors were expressed in malignant and reactive lymphocytes, causing lymphocytes dysfunction. Understanding the heterogeneous expression of co-inhibitory receptors may be critical for individualized therapy in advanced CTCL patients.

SS is a highly malignant CTCL, affecting both the circulatory system and the skin, with a low 5-year survival rate. Borcherding et al [40] used scRNA-seq and scTCR-seq analyses on malignant $CD4^+$ T cells ($CD3^+CD4^+CD5^{bright}SSC^{hi}$, SS cells) of SS patients and normal $CD4^+$ T cells ($CD3^+CD4^+CD5^{int}SSC^{int}$) of healthy controls, revealing that *CD26* (*DPP4*) expression was downregulated while *CD70* expression was upregulated in SS cells. And scTCR-seq showed that normal cells had 4007 clonotypes, while 97.3% of SS cells had only 1 single clonotype, *TRBV14* and *TRAV9-2*. Although malignant cells showed a single clonal state, multiple malignant subpopulations were identified, and their different functions were enriched by cell trajectory and pseudo-time analysis. *FOXP3* is an important marker of early SS. Thus, analysis of differential expression of malignant cells and normal cells in different states can predict the clinical stage of CTCLs. A combination of 19 DEGs with artificial intelligence (AI) can achieve 80% accuracy for the diagnosis and staging of CTCL.

cSCC and BCC

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin malignancy and the fatality rate is also second to melanoma. Although it can be cured by surgical resection, up to 4% of cSCCs develop nodal metastases. So far, no effective medication is available for this disease. In search of therapeutic targets for cSCC, Ji et al [41] used a combination of scRNA-seq with spatial transcriptomics (ST) and multiplexed ion beam imaging (MIBI) to construct single-cell transcriptome profiles and spatial features for revealing cSCC cellular heterogeneity, microenvironment, and immune mechanisms. They identified a cluster of tumor-specific keratinocyte (TSK) unique in cSCC, consisting of 2.7-13.8% of all tumor cells. The marker genes of TSK subpopulations were mainly associated with cellular movement, ECM disassembly, and epithelial-mesenchymal transition (EMT), and other biomarkers such as *VIM* and *ITGA5*, indicating the invasive behavior. In addition, genes linked to type I interferon response, glycolysis, and hypoxia were upregulated in cSCC. ST analysis showed that TSKs residing within a fibrovascular niche in leading edges of tumors were enriched in cancer-associated fibroblast (CAF) and endothelial transcripts. As for the immune landscape, despite the large differences in spatial composition, CD8 T cells, Tregs, macrophages, and CD4 T cells are highly correlated. They also observed co-localization of Tregs and CD8 T cells using MIBI and a positive correlation of the proportion of CD8 T cells with co-localization. In addition, fibroblasts, macrophages, and Tregs were the most abundant at the tumor-stroma border, whereas CD8 T cells and neutrophils were distributed farther away from the tumor, suggesting that Tregs may prevent effector lymphocytes from entering the tumor to suppress the immune response. Finally, scRNA-seq results of a mouse model of the xenograft tumor showed high consistency with human cSCC samples. Identification of TSK-specific co-essential gene network, including *ITGB1*, *FERMT1*, *CD151*, *ARPC2,* and *HSP90B1*, in vivo by CRISPR/Cas9 screening would be helpful to discover potential therapeutic targets for cSCC.

Immunotherapies that block inhibitory checkpoint receptors on T cells have been increasingly used in the treatment of various skin tumors. However, whether the target T cells are derived from existing tumor-infiltrating lymphocyte regeneration or the recruitment of new T cells recruitment is still unclear. Using scRNA-seq and scTCR-seq, Yost et al. [42] comprehensively analyzed tumor-infiltrating T lymphocyte (TILs) phenotypes and clonal dynamics in patients with basal cell carcinoma (BCC) and cSCC before and after anti-PD-1 treatment. The cell map showed significant heterogeneity among malignant cells in BCC patients, but the TME of the same immune cell population was relatively consistent. A total of 577 differentially expressed genes including Ras were identified. The analysis of TILs showed that the clonally expanded cells were highly enriched with exhausted $CD8^+$ T cells and expression of tumor-specific markers, including CD39 and CD103. Anti-PD-1 treatment mainly affects $CD8^+$ T cells. Replacement of the clone pool of exhausted $CD8^+$ T cells with novel expanded TCR clones suggest that the regeneration ability of T cells in tumors is limited, and that the response to checkpoint blockade relies on the expansion of new specific T cell clones just entering tumors. However, whether the source of new T cell clones is from lymph nodes or around the tumor remains unknown.

Miao et al [43] also used scRNA-seq and lineage tracing to study the mechanisms of recurrence after immunotherapy in a mouse model of skin squamous cell carcinoma (SCC). They found that 2% of tumor-initiating stem cells (tSCs) in tumor cells were highly resistant to T cell immunotherapy. tSCs selectively containing CD80 (a surface ligand previously identified on immune cells) could evade immune surveillance to survive immunotherapy, becoming the source of tumor recurrence. *CD80* is activated only in the tSCs subset that responds positively to TGF-β, and its expression can be reduced by blocking TGF-signaling. The CD80 tSCs can directly suppress cytotoxic T cells to attack tumors by binding to cytotoxic T cell antigen-4 (CTLA4). On the contrary, CTLA4- or TGF-β-blocking antibodies, and CD80 inhibition can weaken the function of tSCs to reduce tumor recurrence. This study provides a potential way to improve immunotherapy for SCC.

Other Skin Tumors

Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasm predominantly affecting children and easily invades

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the skin, bones, lungs and other organs. To characterize the cellular and molecular landscape of LCH lesions, Halbritter et al [44] performed scRNA-seq and ATAC-seq on the skin, bone, and lymph node tissues of primary LCH. A wide range of cell heterogeneity was discovered, and 14 subsets with different degrees of differentiation, proliferation, and immune status were identified. The genetic characteristics associated with neoplastic (cell cycle, DNA replication, MYC) and inflammatory (immunity, inflammation, macrophages, DCs) phenotypes were identified in LCH cells. LCH cells displayed both neoplastic and inflammatory characteristics of LCH. Results of this study demonstrated that using SCS technique can define the transcriptomics and epigenomics characteristics of different cell subgroups in LCH, which not only provides new insights of LCH, but also potentiates the development of personalized treatment for LCH.

Perspective

SCS technology has a variety of applications in dermatology, such as aiding in the study of skin development in both healthy and diseased conditions with the precision of single-cell resolution, discovering new subsets and functions of KCs, HFs, and immune cells, elucidating the pathogenesis of various skin diseases, revealing the interaction between pathogenic microorganisms and hosts in cases of infectious dermatoses, and researching the heterogeneity, development, drug resistance mechanism, and prognosis of skin cancers. Although SCS technology has now covered genomics, transcriptomics, and epigenomics, application of genomics and epigenomics in skin research are still rare. Most of the studies are based on single-omics studies. The functional state of cells depends on the complex inter-regulation of different molecules (including DNA, RNA, and proteins). Therefore, simultaneously acquiring multi-omics features of a single cell is necessary for comprehending the "identity". Single-cell multi-omics methods can perform high-throughput analysis in genomics, transcriptomics, epigenomics, and proteomics of single cells at different omics levels. Explication of the relationship between the genomics, epigenetic modification, and gene expression can provide new methods and perspectives for in-depth analysis of the behavior and mechanisms and their relationship with the organism of individual cells in a population of cells, which will play an increasingly essential role in basic research and precision medicine [45].

SCS requires dissociating tissues into single-cell liquid, resulting in the loss of spatial information about where the cells are located, but spatial transcriptomics solves this technical challenge perfectly by integrating high-throughput mRNA analysis of tissue sections with morphological background of histological section [46]. Hence, SCS technology provides a powerful tool for constructing cellular profiles of skin tissues to reveal the mechanisms of complex skin diseases, and to explore new strategies for diagnosis, prevention, and therapy. Undoubtedly, the single-cell multi-genomics sequencing technique can advance our knowledge of both normal and diseased skin, and is a valuable approach for diagnosis and treatment of skin disorders.

Conflict of Interest

None.

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