Skinomics, transcriptional profiling approaches to molecular and structural biology of epidermis

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■ **Abstract**

Skinomics is a field of bioinformatics applied specifically to skin biology and, by extension, to dermatology. Skinomics has been expanding into extensive genome-wide association studies, eg, of psoriasis, proteomics, lipidomics, metabolomics, metagenomics, and the studies of the microbiome. Here, the current state of the field of transcriptomics is reviewed, including the studies of the gene expression in human skin under several healthy and disease conditions. Specifically, transcriptional studies of epidermal differentiation, skin aging, effects of cytokines, inflammation with emphases on psoriasis and atopic dermatitis, and wound healing are reviewed. The transition from microarrays to NextGen sequencing is noted and potential future directions suggested.

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Skinomics is a field of bioinformatics applied specifically to skin biology and, by extension, to dermatology.[1] Easily accessible, skin was one of the first organs analyzed using DNA microarrays.[2] Specifically, Iyer et al. found that cultured dermal fibroblasts respond to signals from serum by inducing wound healing responses.[3] Ever since, skinomics has been expanding into extensive genome-wide association studies, eg, of psoriasis,[4,5] proteomics,[6] lipidomics,[7] metabolomics,[8] metagenomics,[9] and the studies of the microbiome.[10,11] Here, the current state of the field of transcriptomics is reviewed, examining gene expression in human skin under several healthy and disease conditions. For previous reviews, the reader is suggested to check published references.[12,13] Here, the transcriptomic studies related to epidermal differentiation, aging, inflammation with a particular emphasis on psoriasis, wound healing, and melanoma are recapped.

We note that the transcriptional profiling has "evolved" technically, and the earlier studies using microarrays have been largely superseded by RNA sequencing (RNA-seq) methods (Figure 1). The microarrays were limited because they could only asses the transcription of known and well-characterized targets, namely protein-coding genes; the importance of noncoding, ie, short interfering RNAs and long noncoding RNAs, only became accessible and analyzable with RNA-seq approaches. These, on the other hand, are significantly more labor intensive and costly. Moreover, the computer-based analysis techniques are not as fully optimized as they are for the microarray analysis. Whereas the companies producing microarrays have established corresponding integrated workflows from raw data to analysis results, this has not been as easy for the RNA-seq workflow. The first step in this process is to assess the **quality of raw sequencing data**. For example, any low-quality or contaminating sequences must be eliminated in preprocessing steps. Next, the sequences have to be aligned, ie, mapped to reference sequences already existing in databases. The quality of the alignment should be established and, if necessary, corrected in postalignment processing. Once the sequences are aligned to a reference genome, the analysis of the processed data depends on the experiments performed and questions asked. Such analyses are typically performed using in-house solutions provided by laboratory- or institution-specific IT teams. Thus, the standards for quality control, reproducibility, and documentation are hard to find out or missing. Attempts to provide computational frameworks that automate RNA-seq data analysis pipelines have been made,[14,15] but such frameworks are yet to be widely accepted.

Epidermal differentiation

One of the most fruitful uses of transcriptomics concerns the mechanisms and regulations of epidermal differentiation. Starting with the seminal work comparing the transcription profiles of the basal and suprabasal layers of the epidermis,[16] Radoja et al. identified the signaling pathways specific for the 2 populations. They found 2 previously unknown paracrine and 1 juxtacrine signaling pathway operating between the basal and suprabasal cells. Using specific expression signatures, a novel set of late differentiation markers was identified and their chromosomal loci mapped. The authors also identified a set of markers specific for melanocytes. This work represented a major advancement in identifying the mechanisms of epidermal differentiation.

Calcium gradient in the epidermis is one of the major controllers of epidermal differentiation.[17] Toufighi et al. used integrative and structural network analyses to dissect the calcium-induced differentiation of keratinocytes.[18] Specifically, they looked at the differentiation-associated dynamic changes in formation of protein complexes. The authors suggest that complex assemblies with stable core components, transiently acquiring specific subunits, guide cells into diverse differentiation stages. Another major regulator of epidermal differentiation essential for initiating the differentiation process is the detachment of the basal keratinocytes from the basement membrane.[19] Using comprehensive transcriptional profiling, we defined the changes in keratinocytes specifically caused by detachment from the substratum.[20] In suspended keratinocytes, 762 genes were overexpressed, while 1,427 were overexpressed in attached cells. Detachment induced, as expected, the expression of cornification and desmosomal markers, but also proliferation inhibitors and innate immunity genes. Conversely, the attached cells

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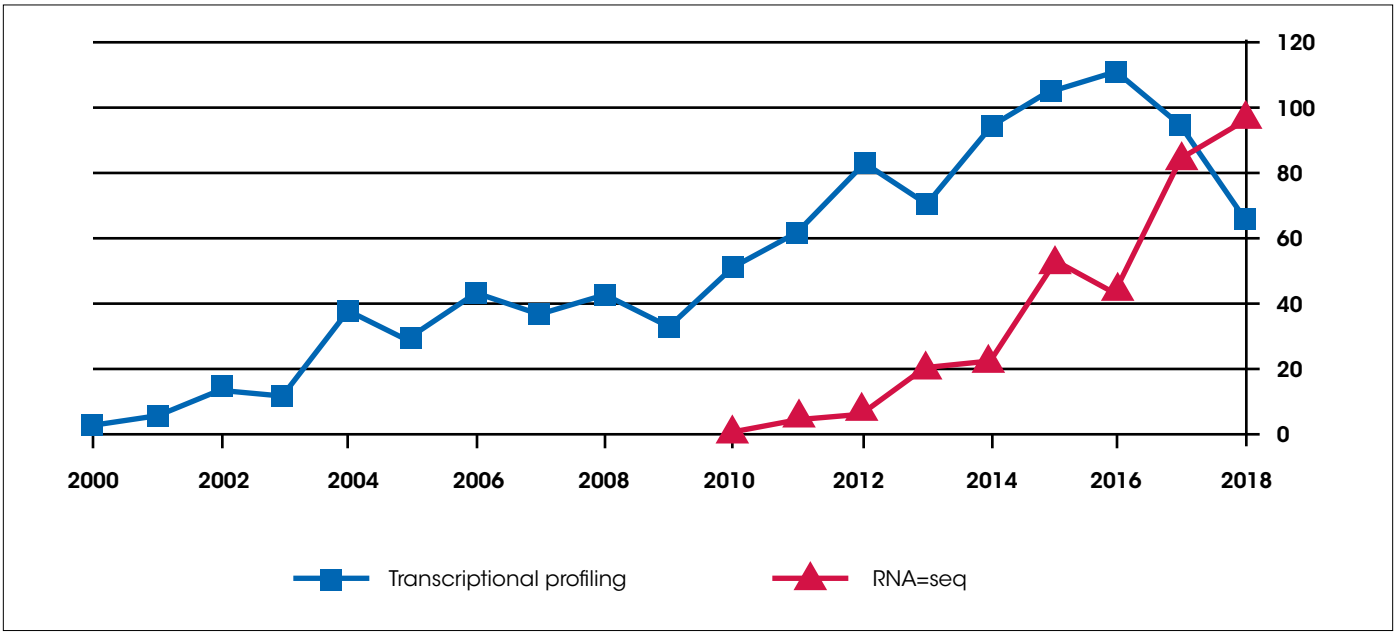

FIGURE 1. The numbers of articles in PubMed with search terms "Skin AND Transcriptional Profiling" and "Skin AND RNA-seq."

overexpressed cell cycle and anchoring proteins but also mitochondrial genes and regulators of apoptosis, both positive and negative. The melanosome trafficking components were also overexpressed in the attached keratinocytes.

The molecular mechanisms and signaling processes that transmit pro-differentiation signals are not fully known, but inhibition of Jun N-terminal kinases (JNK) seems to be a necessary component.[21] Using a JNK-specific inhibitor, we comprehensively defined the JNK-regulated genes in human epidermal keratinocytes. We found that in vitro JNK inhibition induces virtually all aspects of in vivo epidermal differentiation, namely withdrawal from the cell cycle, expression of cornification markers, stratification, and even production of fully formed cornified envelopes. Moreover, inhibition of JNK also induced mitochondrial proteins, histones, and DNA repair enzymes. In parallel, the enzymes of lipid and steroid metabolism, proteins of the diacylglycerol and inositol phosphate pathways, were induced; notably, these have not been previously associated with epidermal differentiation. At the same time, JNK inhibition suppressed the expression of basal cell markers, extracellular matrix proteins, integrins, and components of hemidesmosomes. These effects are specific for inhibitors of JUN because inhibiting ERK and p38 kinases (with JNK, members of the MAPK family of kinases) did not induce differentiation markers.[5]

Among the best-characterized targets of JNK are the AP1 transcription factors, members of the Jun and Fos families.[22] Indeed, the role of the AP1 proteins in epidermal differentiation has been demonstrated in vivo using transcriptional profiling. Specifically, embryonic deficiency of AP1 transcription factor causes a collodion baby phenotype.[23] A specific target of AP1 in keratinocytes is the filaggrin gene, which encodes a major component of the epidermal barrier. Additional targets of c-jun were identified using a dominant-negative c-jun construct in transgenic mice[24] and include cutaneous keratins, filaggrin, filaggrin2, late cornified envelope precursor proteins, hair keratins, and hair keratin-associated proteins. Conversely, the expression of hyperproliferation-associated epidermal keratins Krt6a, Krt6b, and Krt16 was increased. Inactivation of AP1 transcription factor, on one hand, reduces expression of late differentiation markers and is associated with a compensatory increase in early differentiation markers.[24] AP1 deficiency may also be causing an inflammatory response, eg, by activating additional signaling pathways.

One of the known restraining effects on epidermal differentiation are the retinoids, used widely in dermatologic practice.[25] Retinoids affect transcriptional control through nuclear receptors, a family of transcription factors that bind small molecules, hormones, vitamins, and lipids, then as homodimers or heterodimers bind specific DNA sites directing transcription from nearby promoters.[26] The transcriptional effects of retinoic acid on epidermal keratinocytes in culture include, as expected, suppression of the protein markers of cornification.[27] Additionally, the expression of genes responsible for biosynthesis of epidermal lipids, long-chain fatty acids, cholesterol, and sphingolipids are also suppressed. Retinoic acid regulates many genes associated with the cell cycle and apoptosis. The response to retinoic acid is fast; hundreds of genes are regulated within the first hour.[27]

The thyroid hormone receptor belongs to the same transcription factor family.[28] Keratinocyte treatment with thyroid hormone regulates many of the genes associated with epidermolysis bullosa, including integrin beta 4, plectin, collagen XVII, MMP1, MMP3, and MMP14.[29] Glucocorticoids, anti-inflammatory agents widely used in dermatology, also act via their nuclear receptors. In epidermal keratinocytes, glucocorticoids inhibit the interferon gamma (IFNγ) pathway and wound healing but also promote terminal differentiation.[30]

One of the best-studied regulators of epidermal differentiation is protein p63, a transcription factor member of the p53 oncogene family.[31,32] Using transcriptional profiling, Kouwenhoven et al. demonstrated extensive and dynamic interactions of p63 with enhancers in differentiating keratinocytes.[33] The p63-bound enhancers bind additional transcription factors, which then cooperate with p63 to regulate gene expression. A core set of ~1,600 conserved DNA sites, distributed among enhancers and super-enhancers, are occupied by p63 in keratinocytes both human and murine.[34]

We found that ephrins and their receptors, in particular receptor Eph-2B, acting as an extracellular ligand, induce differentiation markers in epidermal keratinocytes.[35] EphB2 induced expression of keratins KRT1 and KRT10, Small Proline-Rich Repeats proteins (SPRRs), desmosomal proteins, and inhibitors of cell cycle. Simultaneously, EphB2 suppressed basal layer markers, integrins, and cell cycle proteins. Unexpectedly, we found similar effects of ephrin EFNA4. Specifically EFNA4, but not EphB2, induced synthesis of lipid metabolism proteins and also markers of epidermal differentiation. The effects of EphB2 were delayed relative to those of EFNA4.

Paul Khavari and his team at Stanford initiated and then greatly expanded the studies of the noncoding transcriptome and its role in epidermal differentiation.[36] For example, they found that a long noncoding RNA (lncRNA), in cooperation with MAF:MAFB transcription factors, regulates keratinocyte differentiation. Specifically, ANCR and TINCR lncRNAs acted upstream from MAF:MAFB; in turn, MAF:MAFB bound to genes encoding epidermal differentiation transcription factors, including GRHL3, ZNF750, KLF4, and PRDM1.[36]

More recently, important epigenetic regulators of epidermal differentiation have been characterized. Cavazza et al. found that the enhancers and super-enhancers are differentially occupied by transcription factors in transit-amplifying and stem cells, suggesting that enhancers and super-enhancers are responsible for the differentiation-specific transcriptional changes.[37]

Chen et al. identified grainyhead-like 2 transcription factor inhibits expression of the genes clustered at the epidermal differentiation complex, EDC, at chromosome 1q21.[38] Epigenetically, grainyhead-like 2 overexpression inhibited recruitment of histone demethylase and enhanced the level of histone 3 Lys 27 trimethylation in EpiEDC gene promoters enrichment at these promoters.

Epigenetic regulation is also present in epidermal appendages. For example, ectodysplasin A regulates transcription via the SWI/SNF nucleosome remodeling complex, which is necessary for appendages formation during development.[39] Interestingly, noninvasive appendage transcriptome analysis can be used for easy and convenient metabolic analyses; for example, hair follicle transcriptome comparisons from pre and post high-intensity interval training exercise demonstrated enrichment of genes associated with energy metabolism, cell proliferation, and cytokine-cytokine interaction.[40] We can expect novel additional uses for analysis of transcriptomic changes detected in the hair follicles.

Skin aging

Skin aging is an enduring problem in dermatology and cosmetology. Arguably, a major causation of skin aging is the effects of ultraviolet (UV) light. We and others have analyzed the transcriptional effect of UV light on keratinocytes.[41] We could distinguish 3 waves of changes in gene expression, early 0.5–2 hours, medial 4–8 hours, and late 16–24 hours after illumination. The early wave contains transcription factors that change cell phenotype from a fast-growing cell to a paused, activated cell. The second wave contains secreted growth factors, and cytokines and chemokines, as keratinocytes endeavor to alert the surrounding tissues to the UV damage. The late wave contains differentiation markers, as keratinocytes terminally differentiate and die, removing a carcinogenic threat. UV irradiation also induced the expression of mitochondrial proteins to provide additional energy, and the enzymes that nucleotide triphosphates, which synthesize additional raw materials for DNA repair.[41] Specific analysis of the UVA-induced transcriptome changes in dermal fibroblasts by Zheng et al.[42] revealed that genes altered by UVA irradiation involved biological process, cellular component synthesis, molecular function, and metabolic pathway. UV also regulated expression of photoaging-related genes, such as elastin, sprout, cathepsins B, D and K, D, ribose-phosphate diphosphokinase, and phosphoglucomutase.

Focusing on sebocytes of women from 20 to 60 years of age, Makrantonaki et al. identified alterations in pathways related to cell cycle, immune responses, mitochondrial function, oxidative stress, proteolysis, steroid biosynthesis, and phospholipid degradation.[43] These are characteristics of skin aging. Sex-specific transcriptomes of aging skin received special attention.[44,45] Age-related epigenetic changes in DNA methylation were analyzed in 108 skin samples.[46] The methylation data could be used to predict accurately the chronological age of sample donors. Apparently, the loss of epigenetic regulatory fidelity is a key feature of the aging epigenome.

Cytokines and inflammation

In skin, inflammatory and immune responses are orchestrated by the intricate production of and responses to cytokines by the keratinocytes and white blood cells of the immune system.[47,48] Transcription profiling has been extensively used to analyze the responses of both cell types to various immunomodulators.

For example, transcriptional profiling was used to delineate the distinct character and activities of cutaneous dendritic cells in steady state, with the epidermal Langerhans cells contrasting with dermal dendritic cells.[49] Li et al. analyzed the resident memory cells in the epidermis, isolating the CD8(+) permanently resident memory T cells, as well as skin-tropic (CLA(+)) helper T cells, regulatory T cells, and CD8(+) CD103(-) T cells from skin and blood.[50] Their results suggest that skin contains multiple distinct T resident memory cell populations. Studying cytokine expression in chronic spontaneous urticaria, in serum, Chen et al. found a significant increase in Th1-/Th2- and Th17-related cytokines in patients, while the levels of IL-6, IL-10, and IL-13 were significantly higher in patients with acute urticarial than in the patients with chronic spontaneous urticarial.[51] An interesting study of the parallels between atopic dermatitis and reaction to house dust mites discovered significant differences in activation of T cells, refuting the suggestion that reaction to house dust mites can be used as a stand-in for testing for atopic dermatitis susceptibility.[52]

Keratinocytes

Keratinocytes, the predominant cell type in the epidermis, both produce and respond to a plethora of proinflammatory signals, growth factors, cytokines, and chemokines. The transcriptional responses to these signals have been to a great extent defined using transcriptional profiling. For example, interleukin-1 (IL-1) is a proinflammatory and immunomodulatory cytokine in skin, where it plays a role of a sentinel, alerting the body to the presence of bacterial infections or UV damage. IL-1 is a major signaling molecule in inflammatory and in bullous diseases of the skin, especially psoriasis.[53] We defined the transcriptional changes in human epidermal keratinocytes 1, 4, 24, and 48 hours after treatment with IL-1α.[54] IL-1 induced many genes that have antimicrobial function, such as IL-8, IL-19, elafin, C3, and S100A proteins. IL-12 is a cytokine that plays a critical role in immunity by inducing production ofIFNγ and other cytokines. IL-12 blocks the UV light-induced immunosuppression, which is important in cutaneous allergies and inflammation as well as in cancer immunosurveillance.[55] We used large DNA microarrays and defined the transcriptional changes in human epidermal keratinocytes after treatment with IL-12, and in cells treated with both UV light and IL-12.[56] Surprisingly, the transcriptional effects of IL-12 by itself did not rise above background levels. However, pretreatment with IL-12 strongly modulated the transcriptional effects of UV, antagonizing transcriptional regulation of 263 genes. We concluded that in keratinocytes, IL-12 specifically interferes with a subset of transcriptional effects of UV irradiation.[56]

One of the main proinflammatory signals is conveyed by tumor necrosis factor alpha (TNFα). Transcriptional responses to TNFα include not only immune and inflammatory responses but also tissue remodeling, cell motility, cell cycle, and apoptosis. TNFα induces a characteristic large set of chemokines that attract neutrophils, macrophages, and skin-specific memory T-cells. TNFα promotes tissue repair by inducing basement membrane constituents and collagenases. TNFα enhances keratinocyte motility and attachment by inducing integrins and cytoskeleton regulators. Apparently, TNFα initiates responses to injury but also the subsequent epidermal repair.[57] The signal transduction by TNFα proceeds, inter alia, via the NFκB pathway. Using parthenolide, an NFκB-specific inhibitor, we identified the NFκB-dependent set of the TNFα-regulated genes in keratinocytes. Approximately 40% of the TNFα-regulated genes depend on NFκB. Cytokines, apoptosis-related, cornification markers, and antigen presentation proteins are included in the NFκB-dependent group. Most cell cycle, RNA-processing, and metabolic enzymes are not NFκB dependent. Most regulated genes contain consensus NFκB binding sites, except, curiously, the cornification markers.[58]

IFNγ, an immunomodulatory cytokine with antiviral activities, is implicated in many skin diseases, from warts to psoriasis and cancer. We identified the IFNγ-regulated genes in keratinocytes: IFNγ induced tight junction proteins, presumably to deny viruses paracellular routes of infection.[59] IFNγ also induced human leukocyte antigen, cell adhesion, and proteasome proteins to facilitate leukocyte attraction and antigen presentation. IFNγ suppressed the expression of differentiation, presumably to interfere with the epidermal tropism of papillomaviruses. In addition, IFNγ suppressed the expression of many genes responsible for cell cycle, DNA replication, and RNA metabolism, presumably to deny viruses a healthy cell in which to replicate.

We have also determined the transcriptional responses of epidermal keratinocytes to additional growth factor and cytokines, including Oncostatin M,[60] Epidermal Growth Factor (EGF),[61] both ephrins, and their receptors,[35] and Transforming Growth Factor beta (TGFβ).[62]

One of the most common allergens is metallic nickel, a ubiquitous and essentially unavoidable environmental and occupational hazard. Human epidermal keratinocytes are the sentinels for nickel exposure. Therefore, we treated differentiating epidermal keratinocytes grown on air-liquid interface with nickel salts.[63] Functional categories of regulated genes suggest that Ni inhibits apoptosis, promotes cell cycle, and induces synthesis of extracellular matrix proteins and extracellular proteases. Importantly, Ni also regulates a set of secreted signaling proteins, inducing Vascular Endothelial Growth Factor (VEGF), amphiregulin, Placental Growth Factor, GDF15, and BST2, while suppressing IL-18, galectin-3, and Lipopolysaccharide-Induced TNFα Factor. Significant parallels were found between the Ni-regulated genes and the genes regulated by TGFβ, EGF, glucocorticoids, or Oncostatin-M.

Atopic dermatitis

Eczema, or atopic dermatitis, is one of the most common inflammatory diseases, and it was extensively analyzed using transcriptional profiling. For analysis of eczema, the microarray technology gave way to RNA-seq, which allows identification and analysis of noncoding RNAs.[64] Profiling lesional and nonlesional skin of 18 patients with moderate-to-severe atopic dermatitis, Suárez-Fariñas et al. identified inflammatory genes S100A8/A9/A12, CXCL1, and OASL and barrier genes keratin 16 KRT16 and CLDN8. RNA-seq uniquely identified TREM-1, CCL2, CCL3, SIGIRR, and IL-36 isoform genes.

In chronic actinic dermatitis, Lei et al. found 6,889 annotated lncRNAs, 341 novel lncRNAs, and 65,091 mRNAs.[65] As expected, inflammatory and immune response-related pathways were prominent. Differentially expressed lncRNA RP11-356I2.4 probably plays a role in chronic actinic dermatitis by regulating TNFAIP3.

The anti-inflammatory effects of glucocorticoids include regulation of the expected proinflammatory genes, suppressing the expression of essentially all IFNγ-regulated genes, including IFNγ receptor and STAT-1. Glucocorticoids also regulate cell fate, tissue remodeling, cell motility, differentiation, and metabolism. Unexpectedly, they induce the expression of anti-apoptotic genes while repressing pro-apoptotic ones. Glucocorticoids affect wound healing by inhibiting cell motility and the expression of vascular endothelial growth factor, and tissue remodeling and scar formation by suppressing the expression of TGFβ1 and -2 and MMP1, -2, -9, and -10 and inducing TIMP-2. Finally, glucocorticoids promote terminal steps of epidermal differentiation while simultaneously inhibiting markers of early differentiation.[30]

Other inflammatory diseases

Recently Puccetti et al. profiled miRNAs expressed in Behçet's disease, a chronic inflammatory disease with skin lesions.[66] miR-

NAs are key regulators of immune responses, and miRNA signatures specifically associated with Behçet's disease target pathways of TNFα, IFNγ and VEGF-VEGF Receptor signaling.

Shih et al. used 2 large normal skin transcriptomic datasets—one RNA-seq, the other microarray—to define 20 gene signatures in skin.[67] They defined expression signatures for hair follicles; sebaceous, sweat and apocrine glands; keratinocytes; melanocytes; endothelia; muscle; adipocytes; immune cells; and a number of pathway systems. Their resource, SkinSig, was then used to analyze of transcriptomes of 18 skin conditions, showing that a decrease in keratinization and fatty metabolism with age is due to loss of hair follicles and sebaceous glands. This resource can be used to define the functional profile of skin cell types and in interpret disease data. Of all skin diseases, psoriasis arguably has the most deeply studied transcriptome. Psoriasis is an immune-mediated, inflammatory disorder of the skin characterized by hyperproliferation of the epidermis.[68] Led by 2 groups, one in New York, one in Michigan, large-scale transcriptome analyses included hundreds of patients comparing transcriptomes of the psoriatic plaque versus noninvolved skin from the same patient and comparing these versus skin from healthy subjects.[69-72] Because psoriasis received a special attention in this volume (see XXXXXX), here we just summarize a few important points. These 2 teams analyzed lesions from 163 patients and identified 1,233 psoriasis-increased and 977 psoriasis-decreased differentially expressed genes. Increased genes were attributed to keratinocytes and lesion-infiltrating T-cells and macrophages. Prominent induced genes included those induced by IL-1, IL-17A, and IL-20 cytokines. Focusing on the Chinese population, Dou et al. identified 5 key genes in psoriasis: PPARD, GATA3, TIMP3, WNT5A, and PTTG1, suggesting that these may serve as potential biomarkers for the diagnosis and treatment.[71] Focusing on gene coexpression network analysis, Ahn et al. identified 3 network modules including many lncRNAs.[72] The rich lode of data in public repositories made possible very sophisticated molecular analyses of psoriasis not available to other fields. For example, the role of noncoding RNAs as transcriptional regulators in psoriasis, already mentioned,[71,72] is quite significant. Tsoi et al. in the Michigan group detected some 3,000 previously annotated and over a 1,000 novel skin-specific lncRNAs. The novel lncRNAs are enriched for localization in the epidermal differentiation complex genes on chromosome 1.[71] Overall, thanks to transcriptional profiling, several new treatments for psoriasis and psoriatic arthritis have been developed and are in use now. We can expect in the near future patient-specific, individualized therapy based on fine classification of psoriatic type, indeed based on transcriptional profiling.

Melanocytes

Because of the clinical relevance of melanomas in dermatology, several important studies have focused on the transcriptional profiling of melanocytes. In healthy skin, human epidermal melanocytes, as expected, express high levels of genes that function in pigmentation. Comparing the transcriptomes of lightly versus darkly pigmented skin, it was found that many differentially expressed genes are involved in signal transduction. Only one, the putative melanosomal transporter SLC45A2, has a direct function in pigmentation.[73] MIF and ATP6V0B genes are also differentially expressed in dark versus light melanocyte lines.[74] UV light treatment affects ribosomal protein synthesis and the p53 signaling pathway in both types of melanocytes. The 2 types differentially regulate genes associated with inflammatory reactions and cell survival. Interestingly, the keratinocyte-conditioned medium affects signaling pathways, including the mTOR pathway, which is involved in the regulation of cell metabolism, growth, proliferation, and survival.[74]

In a large multi-institutional study, Ko et al. used a 23-gene expression signature to differentiate benign nevi from melanomas.[75] Archival tissue from primary melanomas and melanocytic nevi from 4 independent institutions, including 99 primary metastatic melanomas and 83 melanocytic nevi, was tested with the gene signature. The results of gene expression testing closely correlated with long-term clinical outcomes of patients with melanoma, demonstrating the usefulness of the gene expression signature test.[75] Using meta-analysis of data from The Cancer Genome Atlas, Lopez et al. identified 798 genes differentially expressed between metastatic and nonmetastatic genes, including several critical genes implicated in melanoma metastasis.[76] Regulation of apoptosis and cell proliferation, actin cytoskeleton, focal adhesion, and ubiquitin-mediated proteolysis was the important pathways overrepresented in the differentially expressed genes. The authors have developed a metastasis classifier of high prediction accuracy, with potential clinical applications.[76]

Wound healing

Transcription has been applied to analysis of recalcitrant wounds.[77] Comparison of nondiabetic and diabetic foot skin identified identified SERPINB3 as up-regulated, whereas OR2A4 and LGR5 were down-regulated in diabetic foot skin. In chronic venous ulcers, TGFβ signaling was found to be deficient.[78] Specifically, the TGFβ-inducible transcription factors, GADD45β , ATF3, and ZFP36L1, were suppressed while the genes suppressed by TGFβ (FABP5, CSTA, and S100A8) were induced in nonhealing venous ulcers. Glucocorticoids are known to inhibit wound healing, and mechanisms for this effect have been proposed from the analysis of transcriptional effects of glucocorticoids.[31] To enhance healing of persistent wounds, covering with a bioengineered living cell construct can be quite effective. The mechanism for this healing effect was demonstrated using transcriptional profiling.[79] We found that in venous leg ulcers, a bioengineered living cell construct activates an acute wound healing response, essentially converting a chronic wound into a more acute wound-resembling microenvironment. The changes comprised keratinocyte activation, modulation of the cytokine and growth factor signaling, as well as attenuation of the Wnt/β-catenin signaling pathway. The living cell construct application orchestrated a shift from nonhealing chronic ulcer to an acute healing milieu, resembling that of healing wounds.

Conclusions and predictions

The current state of the field of transcriptomics, the studies of the gene expression in human skin under healthy conditions and several disorders, is considerably advanced. The methodology advanced from early microarray approaches to more complete and more informative next generation sequencing.[80] Details of transcriptional

profiles of many skin-resident cell types have been identified and catalogued.[81] These data are easily accessible in public databanks.[82] Specifically in dermatology, we are on the threshold of "diagnosis by numbers,"[83] given that molecular signatures of a growing number of skin diseases have been defined.[84] The next major step, optimistically, will be a move to personalized dermatology, with transcriptomic-led individualized treatment.

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