Chapter 7

Application of Bioinformatics Methodologies in the Fields of Skin Biology and Dermatology

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Additional information is available at the end of the chapter

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Abstract

Bioinformatics is a research field that uses computer-based tools to investigate life sciences questions employing "big data" results from large-scale DNA sequencing, whole genomes, transcriptomes, metabolomes, populations, and biological systems, which can only be comprehensively viewed *in silico*. The epidermis was among the earliest targets of bioinformatics studies because it represents one of the most accessible targets for research. An additional advantage of working with the epidermis is that the sample can even be recovered using tape stripping, an easy, noninvasive protocol. Consequently, bioinformatics methods in the fields of skin biology and dermatology generated a fairly large volume of bioinformatics data, which led us to originate the term "skinomics." Skinomics data are directed toward epidermal differentiation, malignancies, inflammation, allergens, and irritants, the effects of ultraviolet (UV) light, wound healing, the microbiome, stem cells, etc. Cultures of cutaneous cell types, keratinocytes, fibroblasts, melanocytes, etc., as well as skin from human volunteers and from animal models, have been extensively experimented on. Here, we review the development of the skinomics, its methodology, current achievements, and future potentials.

Keywords: epidermal differentiation, inflammation, microbiome, noninvasive, psoriasis

1. Introduction: both wide angle and focused view of skin biology

Bioinformatics is an umbrella term for a wide range of methodologies, studies that generate large datasets [1]. The term refers to the methodology, rather than a subject matter (akin to "microscopy"). Omics techniques, rather than focusing on a single protein, gene, metabolite,

microorganism, etc., comprehensively deal with the entire collection of all proteins, the whole genome, the complete metabolic array, or the full microbiome of a given biological system. "Omics" methodology has reached nowadays full maturity and recognition of the research community. The methodology comprises very large datasets that require sophisticated *in silico* analyses. Accordingly, it uses, on the one hand, large central databanks, repositories of raw and "pre-processed" data, and complex suites of analysis programs developed by multidisciplinary teams that include statisticians, graphic designers, etc., and on the other hand, many individual laboratories and groups providing discrete pieces of the large "omics" puzzles and applying the algorithms to their specific objectives [2].

Bioinformatics approaches received a major impetus with the development of "omics" techniques. Arguably, DNA microarrays are the most widely used omics technology [3]. In microarrays, the DNA probes are immobilized on solid supports, and the samples, such as total bulk DNA or RNA from the specimen, are labeled and then hybridized to the arrays in order to measure individual genes. Requiring only minute amounts of input DNA or RNA, such microarrays probe simultaneously, in hugely parallel experiments, many genes, for example, all the genes in the human genome. These experiments create very large volumes of data. Strangely enough, microarrays allow not only a very broad but also a very detailed insight into the biological function, mechanisms, and diseases of interest to dermatology. Microarrays empower us to see both the "forest and the trees."

Bioinformatics is a very rapidly developing science that constantly improves its methodology, microarrays, sequencing aparati, data repositories, hardware, and software. To keep up with the field, we have found very useful the special database issue of the *Nucleic Acids Research* [4], which is published every January. In these issues, we find description of the functions and roles of various data repositories. Another invaluable resource is the *Bioconductor* [5] an ever-expanding collection of bioinformatics algorithms developed by computer scientists and programmers from all over the world. The *Bioconductor* analysis packages are freely available to all. They are well described and annotated, and usually the program developers are helpful in troubleshooting and even extensive hand-holding.

Directly accessible, skin was among the first organs analyzed using omics approaches. As a result, dermatology was one of the first medical disciplines to welcome and support omics results. Name "skinomics" has been proposed to designate specifically the bioinformatics studies in dermatology and skin biology [6]. The objectives of skinomics are to provide, enlarge, and buildup our knowledge of skin biology, to improve function of the healthy skin, and to assist in treating pathological skin conditions.

Skinomics studies focused to a significant extent, understandably, on skin cancers [6, 7]. For example, melanoma has been arguably the most studied skin disease. Microarray analyses identified markers of melanoma progression and of its metastatic potential. Similar studies targeted basal and squamous cell carcinomas. Specific for dermatology, noninvasive method using simple tape stripping can provide adequate material for transcriptional profiling of melanoma, psoriasis, and other skin diseases. The molecular changes in psoriatic plaques, that is, differences between uninvolved and involved skin and interestingly, the healed lesions of psoriatics, have also been defined using very large cohorts of patients. The psoriatic patients

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analyzed using this technology by now number in several hundreds [8]. Cutting-edge state-of-the-art international skinomics studies, involving almost 20 different countries, have characterized many of the psoriasis susceptibility loci in the human genome, and identified the genes with putative roles on the pathology of this disease. These genes represent potential targets for intervention [8, 9]. DNA microarrays have been used to follow the course of psoriasis treatment and to predict responses or resistance to specific treatment modalities. The characteristic changes in the microbiomes of psoriasis and of atopic dermatitis patients have been correlated with the progression of each disease.

Arguably the most frequent and continual methodology in skinomics is the use of DNA microarrays, such as those from Affymetrix and Illumina. The DNA microarrays are a perfect medium because they simultaneously measure the expression of the entire genome [10]. Printed cDNA arrays, originated by Brown at Stanford [11], are often homemade, inexpensive, and two color, that is, compare two samples on the same chip. They are easy to customize in-house for specific applications. The commercial synthetic oligonucleotide microarrays are pricier, but tend to be more reliable. The microarray community has established a set of guidelines known as "MIAMI" rules (minimal information about microarrays) to allow comparisons of data obtained using different microarrays, sample handling techniques, quality of data, etc.

In the field of skin biology, DNA microarrays have been used to identify the genes specific for epidermal stem cells. Moreover, the transcriptional changes occurring during the process of epidermal differentiation have been characterized. The consequences of epidermal and barrier disruption have been defined. Importantly, cultures of epidermal keratinocytes *in vitro* have been used by our group as well as many others in many studies because these cells respond to ultraviolet (UV) light, hormones and vitamins, inflammatory and immunomodulating cytokines, chemokines and growth factors, environmental toxins, microbes, physical injury, etc.

As the medical field presses forward in the direction of personalized medicine, we can anticipate that the skinomics approaches will be shortly applied at the bedside, directly to the personalized dermatology practice of the future.

2. Historical perspective

The first microarrays were developed at Stanford University by Dr. Pat Brown and his group [11]. Soon thereafter, they applied this methodology to skin biology [12]. Specifically, using the well-known model to achieve cell cycle synchronization by serum deprivation and then re-introduction of serum to the culture medium, Iyer et al. [12] characterized the timing and choreography of cell cycle gene regulation in synchronized cultures. Unexpectedly, Iyer et al. [12] also found that dermal fibroblasts respond to signals from serum by inducing specifically the wound-healing responses. In retrospect, this result makes perfect sense because dermal fibroblasts are not exposed to serum, except right after wounding, when they are required to mount an appropriate response.

Another Stanford team, the group of Dr. Paul Khavari, was the first to use microarrays in dermatology [13, 14]. They used microarrays to follow the outcomes of gene therapy for junctional epidermolysis bullosa, a lethal genetic skin disorder. The microarray analysis showed that the normal gene expression has not been completely reestablished, although the replacement of the affected gene restored cell growth and adhesion.

Subsequently, melanomas and skin carcinomas have been investigated using microarrays, as have certain inflammatory diseases, such as psoriasis and eczema, as well as responses to allergens and irritants, effects of UV light, skin aging, wound healing, keloid formation, etc. The large body of skinomics data was used in meta-analyses. For example, Dr. Noh and his team were the first to use meta-analysis of microarray data in dermatology [15]. Such meta-analyses include our own work as well [16, 17].

3. Noninvasive sample acquisition

A very important advantage of skin-oriented research is that the samples can be acquired from skin completely noninvasively and almost painlessly. Based on the work of Drs. Morhenn, Benson, and others, it was demonstrated that simple tape stripping provides sufficient quantity and quality of RNA for use in microarrays [18]. The methodology has been useful in studies of psoriasis, melanoma, etc. [19, 20]. Because of the noninvasive access to tissue, dermatology can be expected to lead further advance toward "omics" techniques. These will be directly applicable to the personalized medicine in the future.

4. Epidermal differentiation

Epidermal keratinocytes are "multifunctional" cells: on the one hand, they must differentiate through a tightly choreographed, multistage process in order to create cornified envelopes, unique three-dimensional structures in the stratum corneum (**Figure 1**); on the other hand, keratinocytes must respond to very many extracellular environmental stimuli, ranging from UV light and chemical irritants to bacteria and viruses. Keratinocytes also must communicate with nearby cells including other keratinocytes, melanocytes, dendritic cells, and others, both sending signals to these cells and receiving signals from them. As a result, keratinocytes have a large transcriptome, and they express relatively many genes. To determine which of the expressed genes are inherent to all keratinocytes, specific for the layers of epidermal differentiation and induced extracellularly, we compared the transcriptomes of harvested skin from human subjects in artificially and three-dimensionally cultured, differentiated, and reconstructed epidermis *in vitro* as well as in keratinocytes cultured as monolayers, and in nonkeratinocyte cell types.

Under all conditions, the keratinocytes express many proteases and protease inhibitors. Skin and the three-dimensional constructs, but not keratinocyte monolayers, express epidermal differentiation markers, including filaggrin, involucrin, loricrin, and other cornified envelope components. Skin specifically expresses a large number of transcription factors, cell surface

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receptors, and secreted proteins. Surprisingly, the mitochondrial genes were significantly suppressed in skin, which suggests a low metabolic rate. In culture, keratinocytes amply express the cell cycle and DNA replication machinery, and also integrins and extracellular matrix proteins. These results define the expressed and regulated genes in epidermal keratinocytes [21].

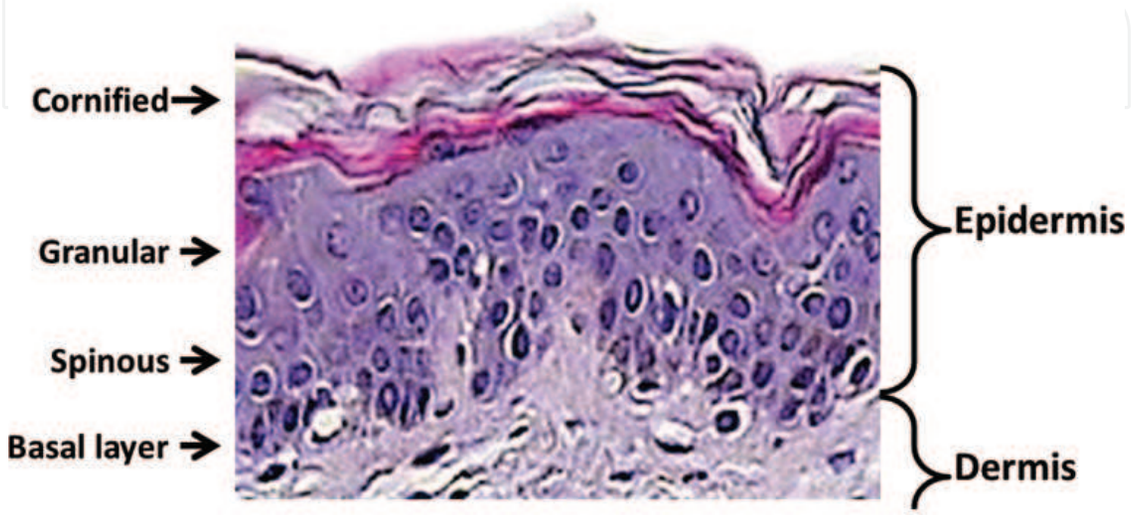

Figure 1. Cross-section through skin. Only the top portion of the dermis is shown, the rest of the dermis extends far below. The layers of the epidermis are marked on the left.

The regulatory circuits that control epidermal differentiation have been a focus of significant research efforts. Inhibition of Jun N-terminal kinase, JNK, in keratinocytes *in vitro* stimulates virtually all aspects of *in vivo* epidermal differentiation, including withdrawal from the cell cycle, cessation of motility, stratification, and production of cornified envelopes [22]. Inhibiting JNK also induces the expression of genes responsible for lipid and steroid metabolism, mitochondrial proteins, and histones. Simultaneously, the transcripts for basal cell markers are suppressed, including those for integrins, hemidesmosomes, and ECM components. We found that in the promoter sequences of JNK-regulated genes, the forkhead family binding sites and the c-Fos binding sites are overrepresented [22].

Vitamin D and calcium promote epidermal differentiation [23]. Specifically, kallikreins, serpins, and c-Fos were found to be vitamin D-responsive genes with roles in epidermal differentiation. Seo et al. [24] identified a subset of calcium-regulated genes in human keratinocytes [24]. Conversely, retinoic acid and its analogs inhibit differentiation of epidermal keratinocytes [25]. Microarray analysis identified Rho as another signaling molecule that suppresses differentiation-associated genes [26]. The papillomavirus type 16, HPV-16, E6 oncoprotein inhibits keratinocyte differentiation and suppresses transglutaminase, involucrin, elafin, and keratins [27]. Different classes of HPVs have different effects on cellular transcription patterns [28].

TGFβ promotes the basal, undifferentiated phenotype in keratinocytes via the SMAD4 transcription factor [29]. Interestingly, TGFβ-induced cell cycle arrest and migration genes via SMAD4, but not the epithelial–mesenchymal transition associated genes; these were not SMAD4-dependent, which suggest that a loss of SMAD4 in human carcinomas may interfere with the tumor-suppression, while maintaining the tumor-promoting responses to TGFβ.

To study epidermal differentiation transcriptome *in vivo*, we have separated the basal and suprabasal layers of skin and compared the transcriptomes of the two cell populations [28]. The human skin samples otherwise discarded after reduction mammoplasty are obtained usually within 2–6 h after surgery. The adipocytes and most of the dermis are physically removed leaving ~0.2 mm of mostly the epidermis. After enzymatic treatment, the epidermis is gently separated and single cell suspension derived using trypsin [16, 30, 31]. Magnetic beads attached to integrin β4 antibody are used to collect basal keratinocytes while the non-adherent, β4-cells represent the suprabasal cell populations. We disrupted the epidermal cells and isolated the RNA by using Trizol reagent. Next we use Qiashredders to homogenize cell extracts, remove DNA using on-column DNAse digestion RNeasy prepare the RNA using kits from Qiagen. As quality control, we visualize 28S and 18S ribosomal bands and check that the OD260/280 spectrophotometric ratio is at least 1.8. In the next step, ~5 μg of RNA is labeled according to the Affymetrix-suggested protocols.

5. The skin microbiome

Skin, our outermost layer, represents the first line of defense against pathogenic microbes. The intimate contact between the skin and the infectious microbial world has been known since the biblical days, was already discussed by Hippocrates, and has been studied for a very long time [32–34]. Microbes were perceived primarily as pathogens, which, fulfilling the Koch's postulates, can cause acne, impetigo, folliculitis, etc. As a result, skin microbes have been treated with disinfectants and antibiotics [35–37]. Knowledge of skin microorganisms was deficient because this knowledge was based on *in vitro* culturing of these microbes. While a few cutaneous bacteria and fungi could be grown in laboratories, the vast majority of micro-organisms known to reside on human skin was, and still is, uncultivatable [38]. Recent advances in large-scale DNA sequencing led to major breakthroughs in defining the cutaneous microbiome. Specifically, the 16S small subunit ribosomal RNA in prokaryotes and 18S in eukaryotes are encoded in genomes of all living organisms; genes encoding these RNAs are closely related so that a set of PCR primers can be used for multi-taxa amplification, providing unambiguous identification of individual species [39].

The major breakthrough in defining the cutaneous microbiome occurred in 2007 when Dr. M. Blaser's laboratory at the New York University, USA, published the molecular analysis of superficial skin bacterial biota of human forearm [40]. In this work, Gao et al. found that the cutaneous microbiome consists predominantly of six bacterial genera, *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Acinetobacter*, and *Finegoldia* were present in all subjects and represented 63% of all DNA clones analyzed. Approximately 8% of clones

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represented previously unknown organisms. Some 300 different bacteria inhabit our skin, although we humans demonstrate remarkable interpersonal variation in our cutaneous microbiota. Different body sites harbor specific microbial patterns that characteristically change in skin diseases [40]. Four phyla, *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, constitute vast majority of skin bacteria, while *Malassezia* dominates the skin fungi (**Figure 2**). The somewhat more complex fungal 18S RNA genes can be used to identify fungi, yeasts and other eukaryotes, and they have been used to confirm the abundance of *Malassezia* species on human skin [41, 42]. Current understanding of the cutaneous microbes has shown that they are, for the most part, commensal and beneficial, useful and protective, and only rarely dysbiosis and infection of pathogens occur.

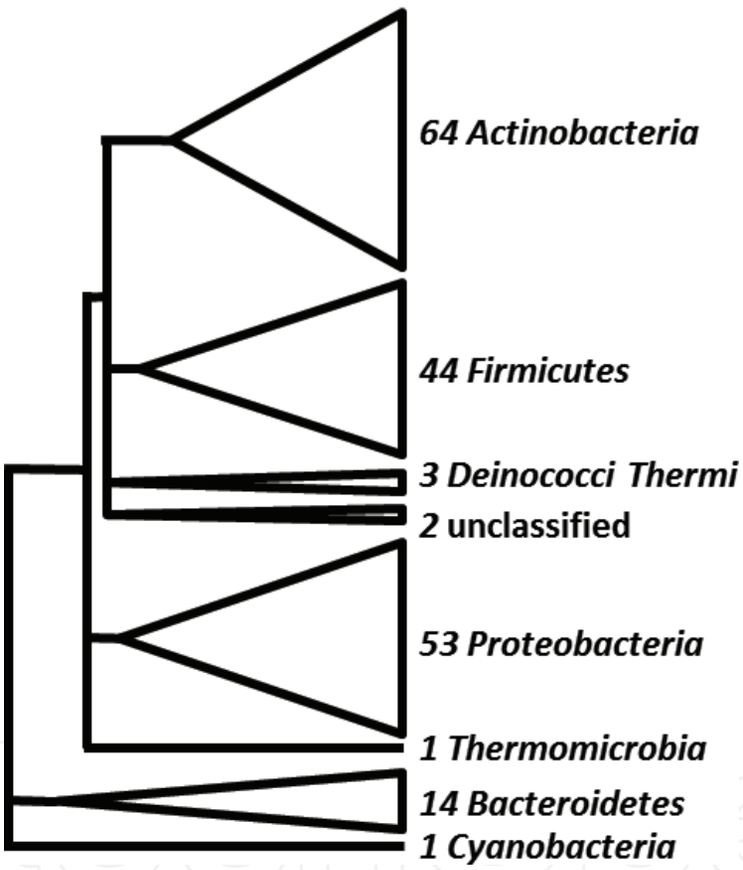

Figure 2. Bacterial populations in the cutaneous microbiota. Note the predominance of *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. The numbers refer to different species, or "operational taxonomic units" detected.

Different body sites harbor different bacterial complexes. In a very significant microbiome analysis, Dr. Segre and her collaborators described microbiomes from 20 different body sites from 10 healthy individuals [43]. From such data, the authors were able to make several important conclusions regarding our cutaneous microbiome. Intrapersonal variation between symmetrical sites, that is, left vs. right forearms was much less than the interpersonal variation. Perhaps unexpectedly, the protected sites, such as inguinal and alar creases, were more related than were the freely exposed sites, such as forearms. Re-sampling resulted in higher intraper-

sonal similarity, suggesting a fairly high consistency of the microbiome during the 4–6 months duration of the study.

The types of the microbiota were classified according to the type of skin as sebaceous, moist, or dry [43]. These sites had different proportions of *Actinobacteria, Firmicytes, Proteobacteria,* and *Bacteroidetes*. In addition to sebaceous/moist/dry, it is possible to construct additional classifications of the skin microbiota: while overall *Corynebacteria* and *Propionibacteria* are the most common genera, *Actinobacteria* are especially common on the UV-exposed sites, glabella, nares, and occiput; these genera are not as common on sun-protected skin. Moreover, the *Proteobacteria* are particularly common on human arms, that is, on axillae, forearm, palms, and the interdigital space. Skin sites that are commonly subject to stretching and flexing, including fingers, toe webs, popliteal and antecubital fossa, inguinal crease, and occiput are particularly rich in *Staphylococci*. The gluteal crease and the toe webs are particularly rich in *Micrococci*. As suggested by Segre et al. [43], the human skin contains multiple and varied niches that are host to multiple and varied microbiota.

6. UV damage

UV light is a major environmental carcinogen. Photodamage of skin results in thinning, wrinkling, keratosis, and ultimately malignancy. Several groups, including ours, have analyzed the transcriptional responses to UV light in human epidermal keratinocytes [44–48]. Keratinocytes respond to UV by inducing a cell repair program to self-repair autonomously. However, the keratinocytes also must protect the underlying organism (**Figure 3**). The early transcriptional changes, that is, in the first two hours after UV illumination, contain expression of transcription factors, signal transducing, and cytoskeletal proteins resulting in a "paused" phenotype. This allows keratinocytes to assess the damage and commence repair functions. After 4–8 h post-irradiation, keratinocytes secrete signaling peptides, growth factors, cytokines, and chemokines; these serve to alert the underlying tissue to the UV damage. Subsequently, 16–24 h after treatment, the cornified envelope proteins are produced, as keratinocytes terminally differentiate. This has two beneficial effects: it boosts the stratum corneum, the protective inert layer of the epidermis, and also removes the cells containing potentially UV-damaged DNA, a carcinogenic threat [44]. The results from several laboratories were quite congruent, especially considering the differences in experimental approaches, countries of origin, and the time frames of the experiments [44–48].

These *in vitro* studies have been followed up by studies of UV-irradiated skin in human volunteers *in vivo* [49, 50]. *In vivo* in skin, markers of keratinocyte activation, such as keratin K6 and S100A proteins, were prominently induced by UV, as were the DNA repair proteins. Interestingly, keratinocytes exposed to gamma or X-rays irradiation produce similar transcriptional changes to those in the UV-treated cells [51, 52], specifically, inducing the genes involved in cell energy metabolism. An interesting study compared samples of lentigines with adjacent sun-exposed skin and with matched samples of sun-protected buttocks skin [53]; genes specifically upregulated in solar lentigo included melanocyte-related genes, genes related to fatty acid metabolism and genes related to inflammation.

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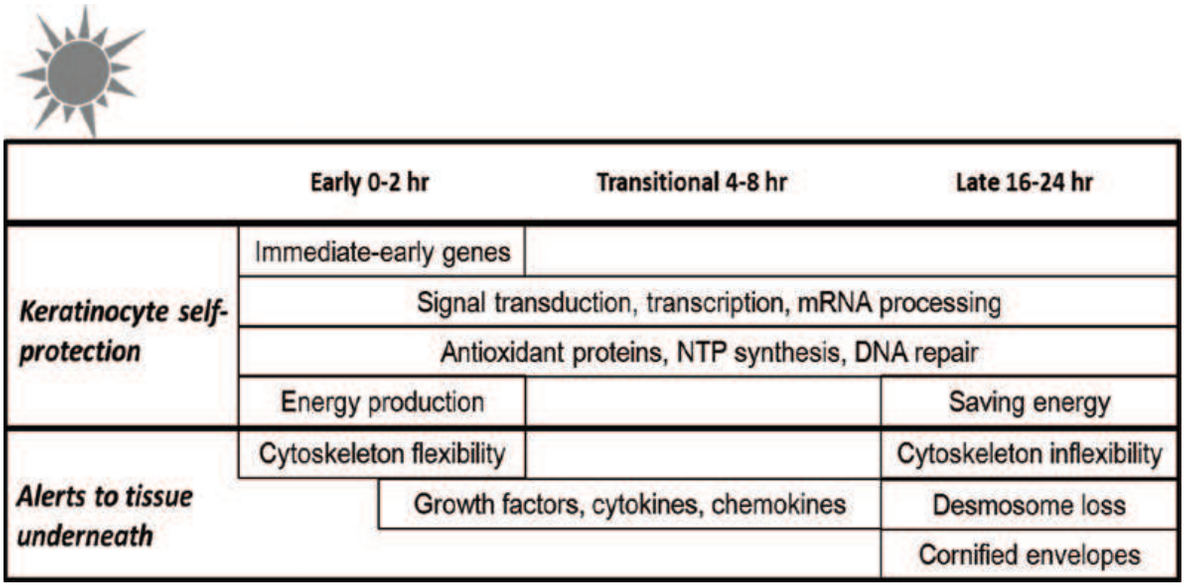

Figure 3. Transcriptional effects of UV light on epidermal keratinocytes. The time course was followed for the first 24 h post-illumination. The UV-treated keratinocytes repair the damage they suffered, as well as alert the underlying tissue that injury has occurred.

7. Skin aging

Skin is the most obvious yardstick of aging. Molecular comparisons of transcriptional profiles of young vs. aged and of sun-protected vs. sun-exposed skin indicate that photoaging and chronical aging, although partly overlapping, have different, characteristic features [54]. Genes associated with skin aging were identified by comparing foreskin keratinocytes from young, 3–4 years of age, old, 68–72 years, subjects [55]. A total 105 genes changed; for example, epidermal differentiation and keratinocyte activation markers were overexpressed in the aged skin, while the immune response, cell cycle, and extracellular matrix associated genes were overexpressed in keratinocytes from young skin. Proteomic profiling using two-dimensional gel electrophoresis to compare young and old foreskin samples identified additional markers of intrinsic aging, including aging-related posttranslational protein modification [56].

One of the hallmarks of aging skin is impaired wound healing. Using microarrays to compare gene expression in wounds of elderly vs. young humans, it was found that the differences seem to be related to regulation by estrogen [57]. These results suggest that estrogen has a profound influence on skin aging in general, and particularly in the context of wound healing. Another hallmark of aging is the graying of hair; analysis of differential gene expressions between pigmented, gray, and white scalp hair follicles identified close to 200 upregulated and as many downregulated genes in human gray hair. As expected, the melanogenesis and structural genes of the melanosome are overrepresented among the regulated genes [58].

8. Skinomics genome-wide association studies

Genome-wide association studies, GWAS, comprise examination of many common DNA polymorphisms in a large population cohort to detect association of polymorphisms with a given disease. Such polymorphisms can point to the genes where disease-causing mutations may map. GWAS are particularly useful in the analysis of diseases, such as psoriasis, which are common and with a strong genetic component. Psoriasis is a hyperproliferative autoimmune skin disease and involves keratinocytes and T cells [9]. A successful GWAS analysis of the psoriasis susceptibility loci in the human genome has been accomplished by an extensive multinational effort (**Figure 4**) and reported in a set of manuscripts [59–66]. A total to 36 loci have been associated with psoriasis in European populations, with additional ones detected in the Chinese population [67, 68]. Other skin diseases, for example, eczema, have also been studied using GWAS; eczema was associated several genetic loci, including major histocompatibility complex (MHC) on chromosome 6 and the epidermal differentiation complex, EDC, on chromosome 1. The human filaggrin gene, known to be associated with eczema, is encoded within the EDC.

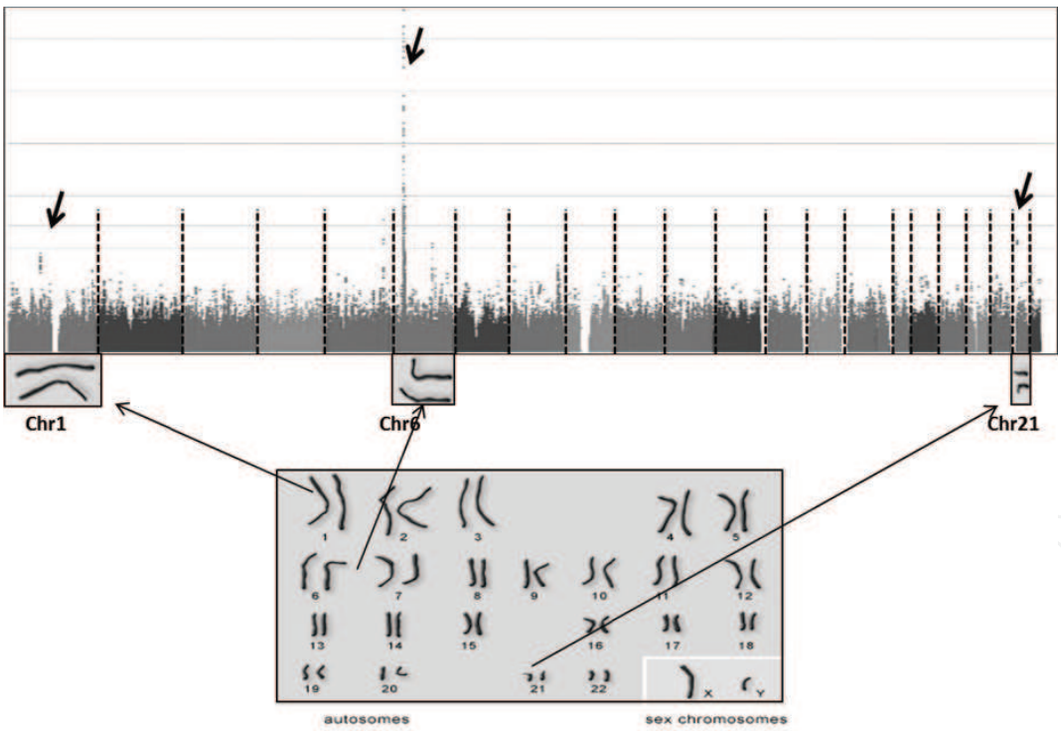

Figure 4. Genome-wide association studies of psoriasis. While many loci have been identified, and additional ones keep being reported, the most prominent ones on chromosomes 1, 6, and 21 are marked with arrows.

Carcinomas and melanoma were also analyzed using GWAS. Basal cell and squamous cell carcinomas have both joint and specific susceptibility loci [69]. Carcinoma GWAS loci are not associated with melanoma risks. GWAS also identified the loci important for human skin pigmentation [70].

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9. Transcriptional studies of melanoma

Melanoma is one of the most aggressive human cancers, which is why it was among the earliest cancers studied using microarrays, already 1 year after the first report of cDNA microarrays [11, 71]. Great amount of attention has been devoted to melanoma diagnosis and the natural history of its progression. Multiple studies compared healthy melanocytes to melanoma tumor cells, other studies examined the differences between melanoma lines that differ in their metastatic potential [71–82]. Some of the differentially expressed genes are, as expected, encoded in the chromosomal regions identified by GWAS as commonly altered in melanomas. Osteopontin, for example, was identified as an overexpressed marker of invasive melanoma.

Microarrays have also been used to compare the transcriptomes of benign moles and melanomas. The metastatic samples exhibited two distinct patterns of gene expression, and these were similar to the microdissected nodular vs. flat components of large primary melanomas [74]. The epigenetic characteristics of melanomas, such as differences in the genome, its methylation, and the expression of miRNAs have also received significant attention. The abundance of primary, raw skinomics data enabled meta-analysis approaches, which consolidated the findings from many studies and established a specific "melanoma signature" gene sets; these could become potentially useful in detection, classification, and outcome prediction for melanomas [83].

10. Wound healing studies

A very active area of skinomics research relates to wound healing, a multi-step process involving coordinated and interacting regulatory pathways [84–86]. For example, we have demonstrated that microarrays can be utilized at the bedside to guide surgical debridement of non-healing wounds [87]. Specifically, the healing edges of ulcers express keratinocyte markers, whereas the non-healing ones express the dermal and inflammatory markers [88]. Diabetic foot ulcers and chronic venous ulcers have distinct transcriptional profiles [88, 89]. The microbiomes of chronic wounds and wound healing have also received attention [90].

11. Inflammation, cytokines, and chemokines

Psoriasis has been linked with multiple cytokines and chemokines, including IL-1, IL-12, IL-17, tumor necrosis factor (TNF)α, interferon (INF)-γ, and oncostatin M. Therefore, the transcriptional effects of these signaling proteins on epidermal keratinocytes in culture have been studied extensively [91–97]. The transcriptional profiling studies of the effects of corticosteroids, anti-inflammatory agents, demonstrated their inhibition of the TNFα, IFN-γ, and IL-1 pathways [98]. However, the anti-inflammatory effects of corticosteroids have characteristic choreography and phasing: the earliest are the anti-TNFα effects, clear already in the first

hour. These are followed by the anti-IL-1 effects, peaking between 24 and 48 h. Finally, the anti-IFN-γ effects occur later, at 72 h.

In separate studies, comparisons of eczema and psoriasis showed that the expression of many antimicrobial proteins of the innate immune response genes are relatively decreased in eczema, which could explain the increased susceptibility to infection in eczema than in psoriasis [99–103].

12. Psoriasis transcriptome

The studies of psoriasis, a paradigmatic skin inflammatory disease, have provided several hundreds of patient samples from several laboratories [8, 9]. This provided data for meta-analysis of the psoriatic transcriptome [16]. Microarray data can be obtained from annotated and curated repositories. The two main data repositories that collect and annotate transcription profiling using microarrays are NIH-GEO [104] and ArrayExpress [105]. The two largely overlap, but have a few differences, that is, studies present in one but not the other collection. Additional datasets exist in proprietary databases, but we found that searching these is time consuming and usually unproductive.

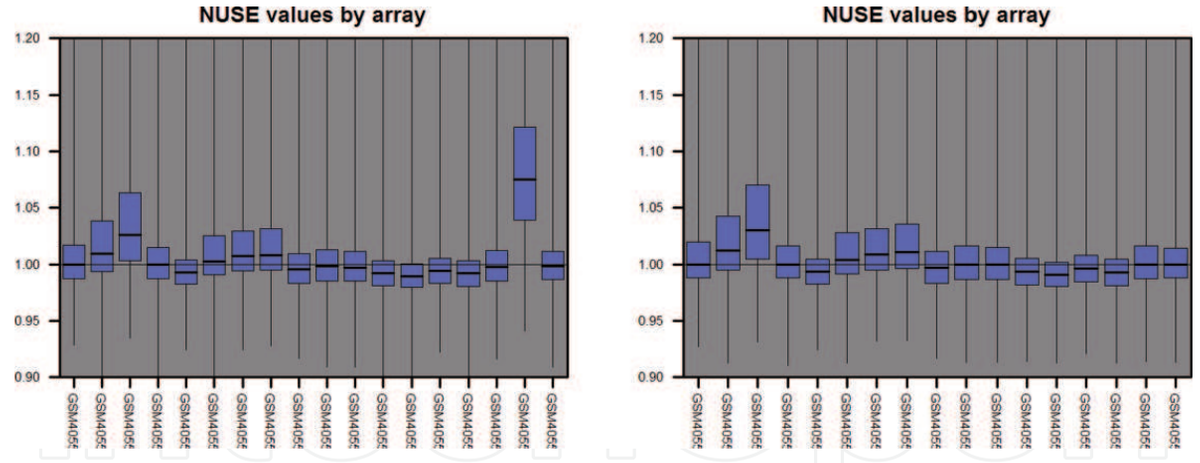

Figure 5. Quality control features of RMAExpress. Before removing a "bad" chip, left and after right.

We searched GEO Datasets for the key term "Psoriasis" and selecting "Homo sapiens" as the organism; from the results, we selected nine studies that met our criteria, namely comparing lesional and nonlesional psoriatic skin. These nine studies included seven Affymetrix-based and one each Sentrix and Illumina microarrays, with a combined total of 645 samples. The Affymetrix .CEL or .TXT files from these studies were downloaded and unzipped, then log2 transformed. Datasets obtained were combined and analyzed using RMAExpress for quality control [106]. RMAExpress is a program that can compute gene expression values from

Affymetrix chips using the Robust Multichip Average protocol; it is available [107]. RMA can also perform chip quality assessments.

We cannot overemphasize the importance of data normalization and selection, when conducting meta-analyses. One of the most is important, but often overlooked task is to perform quality control on the microarrays deposited in the databanks. The quality control graphs can be viewed in the RMAExpress program, the best routine being the normalized unscaled standard error (NUSE). The gene chips with NUSE medians more than 5% different from other chips should be ignored (**Figure 5**) [31]. These microarrays chips usually stem from poor quality input RNA.

In the case of non-Affymetrix studies, the simplest approach is to download the TXT data files directly from PubMed [108]. Conveniently, downloading all compressed files into same directory allows batch uncompressing of TXT files, which can be opened using Excel for further analysis. We often use AddIns DataLoader [109] in order to assign gene annotations to the expression data. A considerable stumbling block for meta-analysis is the merging of data from different microarray platforms. The difficulties include different number of genes on different platforms, different levels of redundancies in probing the genes, and inappropriate differences in identifying genes.

To harmonize gene IDs for various platforms we find useful BioMart [110], the downloaded files can be opened using Excel. We also find very useful the AddIns DataLoader [109] for combining similar data from different spreadsheets. Where the smaller arrays do not value for a given gene, we simply added 1. This does not affect the subsequent steps of analysis.

To select differentially expressed genes, we use RankProd, a nonparametric method [30]. The RankProd method combines different datasets thus increasing the number of differentially expressed genes identified. Here we present a simple simulated RankProd set of commands an imaginary analysis of three datasets with 5 + 5, 6 + 24, and 2 + 2 microarrays (psoriatic lesional + nonlesional [14]):

```
memory.size(max = FALSE) 1
memory.limit(size = 24000)
library (RankProd)
data (Your_txt_file)
n1 <- 5
n2 <- 5
n3 <- 6
n4 <- 24
n5 <- 2
n6 <- 2
cl <- rep(c(0,1,0,1,0,1), c(n1,n2,n3,n4,n5,n6)) 2
```

cl

rownames(Your_txt_file)= Your_txt_file [,1] 3

Your_txt_file = Your_txt_file [,-1]

origin <- c(rep(1,10), rep(2,30), rep(3,4)) 4

origin

RP.adv.out <- RPadvance(Your_txt_file, cl, origin, rand = 100) 5

plotRP(RP.adv.out, cutoff = 0.01) 6

topGene(RP.adv.out, cutoff = 0.01)

write.table(topGene(RP.adv.out, num.gene = 1000), row.names = TRUE, col.names = NA, file = "Your_txt_file.txt") 7.

To annotate the differentially expressed genes, we find extremely useful and convenient the program Database for Annotation Visualization and Integrated Discovery (DAVID) [31, 32, 111]. Starting with the uploaded list, DAVID make available "tables" containing details known about the genes, "charts" which contain over-represented pathways, ontological categories, etc., as well as "clusters" of such categories, eliminating some of the redundancies and overlaps. The transcription factor binding sites in the listed genes can also be evaluated using DAVID, although the oPOSSUM programs are much more comprehensive, sophisticated, and convenient [39, 40, 112]. Many microarray data clustering programs are available; our favorite is MEV [113]. Generally, we did not find them informative for the analysis of data of psoriasis patients.

13. Conclusions

One clear advantage in dermatology over other medical specialties is that in the clinic, the noninvasive, painless sampling using the tape stripping methods can provide high-quality samples for skinomics analysis. Already proven useful in diagnosis of certain diseases, the tape stripping will be also used in the microbiome analyses to provide samples of the cutaneous viruses, bacteria, and fungi, alerting to the presence of pathogens. Similar approaches can detect also cutaneous microbial imbalances. Moreover, disease treatments using microbes may be in our future! We cannot even imagine today the future developments in skinomics. In summary, great strides have been already achieved in skinomics, the omics technology applied in dermatology, and skin biology. Skinomics techniques will eventually provide individualized personalized treatments to dermatology patients. Exciting and wonderful times are ahead.

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