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

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<http://dx.doi.org/10.5772/63799>

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

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

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].

TGF β promotes the basal, undifferentiated phenotype in keratinocytes via the SMAD4 transcription factor [29]. Interestingly, TGF β -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 TGF β .

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 β 4 antibody are used to collect basal keratinocytes while the non-adherent, β 4-cells represent the suprabasal cell populations. We disrupted the epidermal cells and isolated the RNA by using Trizol reagent. Next we use Qias shredders 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 μ g 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 microorganisms known to reside on human skin was, and still is, uncultivable [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

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*, *Firmicutes*, *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.

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 chronological 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].

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 TNF α , IFN- γ , and IL-1 pathways [98]. However, the anti-inflammatory effects of corticosteroids have characteristic choreography and phasing: the earliest are the anti-TNF α 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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References

- [1] Pevsner J. Bioinformatics and Functional Genomics. Wiley-Blackwell; Hoboken, NJ, USA, 2015.
- [2] Gligorijevic V, Przulj N. Methods for biological data integration: perspectives and challenges. *J R Soc Interface*. 2015;112:1–19.. pii:20150571. DOI: 10.1098/rsif.2015.0571.
- [3] Bumgarner R. Overview of DNA microarrays: types, applications, and their future. *Curr Protoc Mol Biol*. 2013;Chapter(22):Unit 22.1. DOI: 10.1002/0471142727.mb2201s101.
- [4] http://nar.oxfordjournals.org/content/vol36/suppl_1/index.dtl [Accessed: 2015].
- [5] <http://www.bioconductor.org/> [Accessed: 2015].
- [6] Blumenberg M. Skinomics. *J Invest Dermatol*. 2005;124(4):viii–x.
- [7] Blumenberg M. Skinomics: past, present and future for diagnostic microarray studies in dermatology. *Expert Rev Mol Diagn*. 2013;(8):885–94. DOI: 10.1586/14737159.2013.846827.
- [8] Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol*. 2014;32:227–55. DOI: 10.1146/annurev-immunol-032713-120225.
- [9] Bowcock AM, Krueger JG. Getting under the skin: the immunogenetics of psoriasis. *Nat Rev Immunol*. 2005;9:699–711.
- [10] Quackenbush J, Hegde P, Qi R, Abernathy K, Gay C, Dharap S, et al. Genomics. Microarrays – guilt by association A concise guide to cDNA microarray analysis. *Science*. 2003;302:240–1.
- [11] Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*. 1995;270:467–70.

- [12] Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JC, et al. The transcriptional program in the response of human fibroblasts to serum. *Science*. 1999;283:83–7.
- [13] Robbins PB, Sheu SM, Goodnough JB, Khavari PA. Impact of laminin 5 beta3 gene versus protein replacement on gene expression patterns in junctional epidermolysis bullosa. *Hum Gene Ther*. 2001;12:1443–8.
- [14] Hinata K, Gervin AM, Jennifer Zhang Y, Khavari PA. Divergent gene regulation and growth effects by NF-kappa B in epithelial and mesenchymal cells of human skin. *Oncogene*. 2003;22:1955–64.
- [15] Noh M, Yeo H, Ko J, Kim HK, Lee CH. MAP17 is associated with the T-helper cell cytokine-induced down-regulation of filaggrin transcription in human keratinocytes. *Exp Dermatol*. 2010;19:355–62. Epub 2009 Jul 8.
- [16] Mimoso C, Lee DD, Zavadil J, Tomic-Canic M, Blumenberg M. Analysis and meta-analysis of transcriptional profiling in human epidermis. *Methods Mol Biol*. 2014;1195:61–97. DOI: 10.1007/7651_2013_60.
- [17] Blumenberg M. Profiling and metaanalysis of epidermal keratinocytes responses to epidermal growth factor. *BMC Genomics*. 2013;14:85. DOI: 10.1186/471-2164-14-85.
- [18] Morhenn VB, Chang EY, Rheins LA. A noninvasive method for quantifying and distinguishing inflammatory skin reactions. *J Am Acad Dermatol*. 1999;41:687–92.
- [19] Wong R, Tran V, Morhenn V, Hung SP, Andersen B, Ito E, et al. Use of RT-PCR and DNA microarrays to characterize RNA recovered by non-invasive tape harvesting of normal and inflamed skin. *J Invest Dermatol*. 2004;123:159–67.
- [20] Wachsman W, Morhenn V, Palmer T, Walls L, Hata T, Zalla J, et al. Noninvasive genomic detection of melanoma. *Br J Dermatol*. 2011;164:797–806. DOI: 10.1111/j.365-2133.011.10239.x. Epub 2011 Mar 25.
- [21] Gazel A, Ramphal P, Rosdy M, De Wever B, Tornier C, Hosein N, et al. Transcriptional profiling of epidermal keratinocytes: comparison of genes expressed in skin, cultured keratinocytes, and reconstituted epidermis, using large DNA microarrays. *J Invest Dermatol*. 2003;121:1459–68.
- [22] Gazel A, Banno T, Walsh R, Blumenberg M. Inhibition of JNK promotes differentiation of epidermal keratinocytes. *J Biol Chem*. 2006;281:20530–41.
- [23] Lu J, Goldstein KM, Chen P, Huang S, Gelbert LM, Nagpal S. Transcriptional profiling of keratinocytes reveals a vitamin D-regulated epidermal differentiation network. *J Invest Dermatol*. 2005;124:778–85.
- [24] Seo EY, Namkung JH, Lee KM, Lee WH, Im M, Kee SH, et al. Analysis of calcium-inducible genes in keratinocytes using suppression subtractive hybridization and cDNA microarray. *Genomics*. 2005;86:528–38. Epub 2005 Aug 9.

- [25] Baron JM, Heise R, Blaner WS, Neis M, Joussen S, Dreuw A, et al. Retinoic acid and its 4-oxo metabolites are functionally active in human skin cells in vitro. *J Invest Dermatol.* 2005;125:143–53.
- [26] Grossi M, Hiou-Feige A, Tommasi Di Vignano A, Calautti E, Ostano P, Lee S, et al. Negative control of keratinocyte differentiation by Rho/CRIK signaling coupled with up-regulation of KyoT1/2 (FHL1) expression. *Proc Natl Acad Sci U S A.* 2005;102:11313–8. Epub 2005 Aug 1.
- [27] Duffy CL, Phillips SL, Klingelhutz AJ. Microarray analysis identifies differentiation-associated genes regulated by human papillomavirus type 16 E6. *Virology.* 2003;314:196–205.
- [28] Thomas JT, Oh ST, Terhune SS, Laimins LA. Cellular changes induced by low-risk human papillomavirus type 11 in keratinocytes that stably maintain viral episomes. *J Virol.* 2001;75:7564–71.
- [29] Levy L, Hill CS. Smad4 dependency defines two classes of transforming growth factor {beta} (TGF-{beta}) target genes and distinguishes TGF-{beta}-induced epithelial–mesenchymal transition from its antiproliferative and migratory responses. *Mol Cell Biol.* 2005;25:8108–25.
- [30] Radoja N, Gazel A, Banno T, Yano S, Blumenberg M. Transcriptional profiling of epidermal differentiation. *Physiol Genomics.* 2006;27:65–78.
- [31] Lee DD, Zavadil J, Tomic-Canic M, Blumenberg M. Comprehensive transcriptional profiling of human epidermis, reconstituted epidermal equivalents, and cultured keratinocytes using DNA microarray chips. *Methods Mol Biol.* 2010;585:193–223.
- [32] Matheson CD, Vernon KK, Lahti A, Fratpietro R, Spigelman M, Gibson S, et al. Molecular exploration of the first-century Tomb of the Shroud in Akeldama, Jerusalem. *PLoS One.* 2009;4:e8319. DOI: 10.1371/journal.pone.0008319.
- [33] Marples MJ. Life on the human skin. *Sci Am.* 1969;220:108–15.
- [34] Kugelmann TP. Skin bacteria and their role in infection. *J Am Med Assoc.* 1966;195:146–7.
- [35] Roth RR, James WD. Microbial ecology of the skin. *Annu Rev Microbiol.* 1988;42:441–64.
- [36] Till AE, Goulden V, Cunliffe WJ, Holland KT. The cutaneous microflora of adolescent, persistent and late-onset acne patients does not differ. *Br J Dermatol.* 2000;142:885–92.
- [37] Fredricks DN. Microbial ecology of human skin in health and disease. *J Investig Dermatol Symp Proc.* 2001;6:167–9.
- [38] Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol.* 2011;9:244–53.
- [39] Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical

- microbiology laboratories. *Clin Microbiol Infect.* 2008;14:908–34. DOI: 10.1111/j.1469-0691.2008.02070.x.
- [40] Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. *Proc Natl Acad Sci U S A.* 2007;104:2927–32. Epub 2007 Feb 9.
 - [41] Paulino LC, Tseng CH, Blaser MJ. Analysis of *Malassezia* microbiota in healthy superficial human skin and in psoriatic lesions by multiplex real-time PCR. *FEMS Yeast Res.* 2008;8:460–71. Epub 2008 Feb 20.
 - [42] Paulino LC, Tseng CH, Strober BE, Blaser MJ. Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. *J Clin Microbiol.* 2006;44:2933–41.
 - [43] Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topographical and temporal diversity of the human skin microbiome. *Science.* 2009;324:1190–2.
 - [44] Li D, Turi TG, Schuck A, Freedberg IM, Khitrov G, Blumenberg M. Rays and arrays: the transcriptional program in the response of human epidermal keratinocytes to UVB illumination. *Faseb J.* 2001;15:2533–5.
 - [45] Sesto A, Navarro M, Burslem F, Jorcano JL. Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *Proc Natl Acad Sci U S A.* 2002;99:2965–70.
 - [46] Murakami T, Fujimoto M, Ohtsuki M, Nakagawa H. Expression profiling of cancer-related genes in human keratinocytes following non-lethal ultraviolet B irradiation. *J Dermatol Sci.* 2001;27:121–9.
 - [47] Takao J, Ariizumi K, Dougherty, II, Cruz PD, Jr. Genomic scale analysis of the human keratinocyte response to broad-band ultraviolet-B irradiation. *Photodermatol Photoimmunol Photomed.* 2002;18:5–13.
 - [48] Howell BG, Wang B, Freed I, Mamelak AJ, Watanabe H, Sauder DN. Microarray analysis of UVB-regulated genes in keratinocytes: downregulation of angiogenesis inhibitor thrombospondin-1. *J Dermatol Sci.* 2004;34:185–94.
 - [49] Enk CD, Shahr I, Amariglio N, Rechavi G, Kaminski N, Hochberg M. Gene expression profiling of in vivo UVB-irradiated human epidermis. *Photodermatol Photoimmunol Photomed.* 2004;20:129–37.
 - [50] Enk CD, Jacob-Hirsch J, Gal H, Verbovetski I, Amariglio N, Mevorach D, et al. The UVB-induced gene expression profile of human epidermis in vivo is different from that of cultured keratinocytes. *Oncogene.* 2006;25:2601–14.
 - [51] Koike M, Shiomi T, Koike A. Identification of skin injury-related genes induced by ionizing radiation in human keratinocytes using cDNA microarray. *J Radiat Res (Tokyo).* 2005;46:173–84.

- [52] Lamartine J, Franco N, Le Minter P, Soularue P, Alibert O, Leplat JJ, et al. Activation of an energy providing response in human keratinocytes after gamma irradiation. *J Cell Biochem.* 2005;95:620–31.
- [53] Aoki H, Moro O, Tagami H, Kishimoto J. Gene expression profiling analysis of solar lentigo in relation to immunohistochemical characteristics. *Br J Dermatol.* 2007;156:1214–23. Epub 2007 Apr 5.
- [54] Robinson MK, Binder RL, Griffiths CE. Genomic-driven insights into changes in aging skin. *J Drugs Dermatol.* 2009;8:s8–11.
- [55] Lener T, Moll PR, Rinnerthaler M, Bauer J, Aberger F, Richter K. Expression profiling of aging in the human skin. *Exp Gerontol.* 2006;41:387–97. Epub 2006 Mar 10.
- [56] Laimer M, Kocher T, Chiocchetti A, Trost A, Lottspeich F, Richter K, et al. Proteomic profiling reveals a catalogue of new candidate proteins for human skin aging. *Exp Dermatol.* 2010;19:912–8. DOI: 10.1111/j.600-0625.2010.01144.x.
- [57] Hardman MJ, Ashcroft GS. Estrogen, not intrinsic aging, is the major regulator of delayed human wound healing in the elderly. *Genome Biol.* 2008;9:R80. DOI: 10.1186/gb-2008-9-5-r80. Epub May 13.
- [58] Peters EM, Liezmann C, Spatz K, Ungethum U, Kuban RJ, Daniltchenko M, et al. Profiling mRNA of the graying human hair follicle constitutes a promising state-of-the-art tool to assess its aging: an exemplary report. *J Invest Dermatol.* 2013;133:1150–60. DOI: 10.038/jid.2012.462. Epub Dec 13.
- [59] Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet.* 2009;41:199–204. Epub 2009 Jan 25.
- [60] Zhang XJ, Huang W, Yang S, Sun LD, Zhang FY, Zhu QX, et al. Psoriasis genome-wide association study identifies susceptibility variants within LCE gene cluster at 1q21. *Nat Genet.* 2009;41:205–10. DOI: 10.1038/ng.310. Epub 2009 Jan 25.
- [61] de Cid R, Riveira-Munoz E, Zeeuwen PL, Robarge J, Liao W, Dannhauser EN, et al. Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. *Nat Genet.* 2009;41:211–5. Epub 2009 Jan 25.
- [62] Strange A, Capon F, Spencer CC, Knight J, Weale ME, Allen MH, et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet.* 2010;42:985–90. Epub 2010 Oct 17.
- [63] Stuart PE, Nair RP, Ellinghaus E, Ding J, Tejasvi T, Gudjonsson JE, et al. Genome-wide association analysis identifies three psoriasis susceptibility loci. *Nat Genet.* 2010;42:1000–4. Epub 2010 Oct 17.

- [64] Ellinghaus E, Ellinghaus D, Stuart PE, Nair RP, Debrus S, Raelson JV, et al. Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. *Nat Genet.* 2010;42:991–5. Epub 2010 Oct 17.
- [65] Sun LD, Cheng H, Wang ZX, Zhang AP, Wang PG, Xu JH, et al. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. *Nat Genet.* 2010;42:1005–9. Epub 2010 Oct 17.
- [66] Huffmeier U, Uebe S, Ekici AB, Bowes J, Giardina E, Korendowych E, et al. Common variants at TRAF3IP2 are associated with susceptibility to psoriatic arthritis and psoriasis. *Nat Genet.* 2010;42:996–9. Epub 2010 Oct 17.
- [67] Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat Genet.* 2012;44:1341–8. DOI: 10.038/ng.2467. Epub 012 Nov 11.
- [68] Julia A, Tortosa R, Hernanz JM, Canete JD, Fonseca E, Ferrandiz C, et al. Risk variants for psoriasis vulgaris in a large case–control collection and association with clinical subphenotypes. *Hum Mol Genet.* 2012;21:4549–57. Epub 2012 Jul 19.
- [69] Nan H, Xu M, Kraft P, Qureshi AA, Chen C, Guo Q, et al. Genome-wide association study identifies novel alleles associated with risk of cutaneous basal cell carcinoma and squamous cell carcinoma. *Hum Mol Genet.* 2011;20:3718–24. DOI: 10.1093/hmg/ddr287. Epub 2011 Jun 23.
- [70] Nan H, Kraft P, Qureshi AA, Guo Q, Chen C, Hankinson SE, et al. Genome-wide association study of tanning phenotype in a population of European ancestry. *J Invest Dermatol.* 2009;129:2250–7. DOI: 10.1038/jid.2009.62. Epub Apr 2.
- [71] DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet.* 1996;14:457–60.
- [72] Dooley TP, Curto EV, Davis RL, Grammatico P, Robinson ES, Wilborn TW. DNA microarrays and likelihood ratio bioinformatic methods: discovery of human melanocyte biomarkers. *Pigment Cell Res.* 2003;16:245–53.
- [73] Hoek K, Rimm DL, Williams KR, Zhao H, Ariyan S, Lin A, et al. Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res.* 2004;64:5270–82.
- [74] Haqq C, Nosrati M, Sudilovsky D, Crothers J, Khodabakhsh D, Pulliam BL, et al. The gene expression signatures of melanoma progression. *Proc Natl Acad Sci U S A.* 2005;102:6092–7. Epub 2005 Apr 15.
- [75] Becker B, Roesch A, Hafner C, Stolz W, Dugas M, Landthaler M, et al. Discrimination of melanocytic tumors by cDNA array hybridization of tissues prepared by laser pressure catapulting. *J Invest Dermatol.* 2004;122:361–8.

- [76] Gallagher WM, Bergin OE, Rafferty M, Kelly ZD, Nolan IM, Fox EJ, et al. Multiple markers for melanoma progression regulated by DNA methylation: insights from transcriptomic studies. *Carcinogenesis*. 2005;26:1856–67. Epub 2005 Jun 15.
- [77] Nambiar S, Mirmohammadsadegh A, Doroudi R, Gustrau A, Marini A, Roeder G, et al. Signaling networks in cutaneous melanoma metastasis identified by complementary DNA microarrays. *Arch Dermatol*. 2005;141:165–73.
- [78] Busam KJ, Zhao H, Coit DG, Kucukgol D, Jungbluth AA, Nobrega J, et al. Distinction of desmoplastic melanoma from non-desmoplastic melanoma by gene expression profiling. *J Invest Dermatol*. 2005;124:412–8.
- [79] Talantov D, Mazumder A, Yu JX, Briggs T, Jiang Y, Backus J, et al. Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin Cancer Res*. 2005;11:7234–42.
- [80] Zhou Y, Dai DL, Martinka M, Su M, Zhang Y, Campos EI, et al. Osteopontin expression correlates with melanoma invasion. *J Invest Dermatol*. 2005;124:1044–52.
- [81] Winnepeninckx V, Van den Oord JJ. Gene expression profiling of primary cutaneous melanoma. *Verh K Acad Geneeskd Belg*. 2007;69:23–45.
- [82] Winnepeninckx V, Lazar V, Michiels S, Dessen P, Stas M, Alonso SR, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J Natl Cancer Inst*. 2006;98:472–82.
- [83] Liu W, Peng Y, Tobin DJ. A new 12-gene diagnostic biomarker signature of melanoma revealed by integrated microarray analysis. *PeerJ*. 2013;1:e49. DOI:10.7717/peerj.49. Print 2013.
- [84] Tomic-Canic M, Brem H. Gene array technology and pathogenesis of chronic wounds. *Am J Surg*. 2004;188:67–72.
- [85] Nuutila K, Siltanen A, Peura M, Bizik J, Kaartinen I, Kuokkanen H, et al. Human skin transcriptome during superficial cutaneous wound healing. *Wound Repair Regen*. 2012;20:830–9. DOI: 10.1111/j.1524-475X.2012.00831.x. Epub 2012 Oct 19.
- [86] Deonarine K, Panelli MC, Stashower ME, Jin P, Smith K, Slade HB, et al. Gene expression profiling of cutaneous wound healing. *J Transl Med*. 2007;5:11.
- [87] Brem H, Stojadinovic O, Diegelmann RF, Entero H, Lee B, Pastar I, et al. Molecular markers in patients with chronic wounds to guide surgical debridement. *Mol Med*. 2007;13:30–9.
- [88] Charles CA, Tomic-Canic M, Vincek V, Nassiri M, Stojadinovic O, Eaglstein WH, et al. A gene signature of nonhealing venous ulcers: potential diagnostic markers. *J Am Acad Dermatol*. 2008;59:758–71. DOI: 10.1016/j.jaad.2008.07.018. Epub Aug 20.
- [89] Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest*. 2007;117:1219–22.

- [90] Grice EA, Segre JA. Interaction of the microbiome with the innate immune response in chronic wounds. *Adv Exp Med Biol*. 2012;946:55–68.
- [91] Finelt N, Gazel A, Gorelick S, Blumenberg M. Transcriptional responses of human epidermal keratinocytes to Oncostatin-M. *Cytokine*. 2005;31:305–13.
- [92] Molenda M, Mukkamala L, Blumenberg M. Interleukin IL-12 blocks a specific subset of the transcriptional profile responsive to UVB in epidermal keratinocytes. *Mol Immunol*. 2006;43:1933–40. Epub 2006 Feb 8.
- [93] Gazel A, Rosdy M, Bertino B, Tornier C, Sahuc F, Blumenberg M. A characteristic subset of psoriasis-associated genes is induced by oncostatin-M in reconstituted epidermis. *J Invest Dermatol*. 2006;126:2647–57.
- [94] Banno T, Gazel A, Blumenberg M, Adachi M, Mukkamala L. Effects of tumor necrosis factor- α (TNF α) in epidermal keratinocytes revealed using global transcriptional profiling. *J Biol Chem*. 2004;279:32633–42. Epub 2004 May 15.
- [95] Banno T, Gazel A, Blumenberg M. The use of DNA microarrays in dermatology research. *Retinoids*. 2004;20:1–4.
- [96] Banno T, Adachi M, Mukkamala L, Blumenberg M. Unique keratinocyte-specific effects of interferon-gamma that protect skin from viruses, identified using transcriptional profiling. *Antivir Ther*. 2003;8:541–54.
- [97] Haider AS, Peters SB, Kaporis H, Cardinale I, Fei J, Ott J, et al. Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. *J Invest Dermatol*. 2006;126:869–81.
- [98] Stojadinovic O, Lee B, Vouthounis C, Vukelic S, Pastar I, Blumenberg M, et al. Novel genomic effects of glucocorticoids in epidermal keratinocytes: inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation. *J Biol Chem*. 2007;282:4021–34. Epub 2006 Nov 9.
- [99] Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, et al. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J Immunol*. 2003;171:326–9.
- [100] Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB, Leung DY. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol*. 2003;112:1195–202.
- [101] de Jongh GJ, Zeeuwen PL, Kucharekova M, Pfundt R, van der Valk PG, Blokx W, et al. High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis. *J Invest Dermatol*. 2005;125:1163–73.

- [102] Ogawa K, Ito M, Takeuchi K, Nakada A, Heishi M, Suto H, et al. Tenascin-C is upregulated in the skin lesions of patients with atopic dermatitis. *J Dermatol Sci.* 2005;40:35–41.
- [103] Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Whynot J, Novitskaya I, Cardinale I, et al. Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis. *J Allergy Clin Immunol.* 2007;119:1210–7.
- [104] <http://www.ncbi.nlm.nih.gov/pubmed/> [Accessed: 2015].
- [105] <http://www.ebi.ac.uk/arrayexpress/> [Accessed: 2015].
- [106] Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A.* 1998;95:14863–8.
- [107] <http://rmaexpress.bmbolstad.com/> [Accessed: 2015].
- [108] <http://www.ncbi.nlm.nih.gov/pubmed/> [Accessed: 2015].
- [109] <http://www.add-ins.com> [Accessed: 2015].
- [110] <http://www.biomart.org/biomart/martview/39658a1f84f2d1822db6e184a5cc356e/> [Accessed: 2015].
- [111] <http://david.abcc.ncifcrf.gov> [Accessed: 2015].
- [112] <http://www.cisreg.ca/cgi-bin/oPOSSUM/opossum/> [Accessed: 2015].
- [113] <http://www.tm4.org/> [Accessed: 2015].