The American Journal of Surgery®

The American Journal of Surgery 188 (Suppl to July 2004) 67S–72S

Gene array technology and pathogenesis of chronic wounds

Marjana Tomic-Canic, Ph.D.[a,*], Harold Brem, M.D.[b]

[a]*Departments of Dermatology and Microbiology, New York University School of Medicine, 550 First Avenue, MSB 234, New York, New York 10016, USA*

[b]*Department of Surgery, Columbia University College of Physicians & Surgeons, New York, New York, USA*

Abstract

Many of the limitations in treatment of chronic wounds are based on lack of knowledge of the molecular mechanism(s) of wound healing. Furthermore, diagnostic tools in wound healing are still primarily macroscopic, visual, and histologic. Thus, by understanding mechanisms of wound healing at a molecular level, new treatments can be designed, prevention programs developed, and a better understanding of current treatments provided. The ability to methodically analyze the expression patterns of thousands of genes simultaneously allows for identification of groups of molecular defects that lead to chronic inhibition of the wound-healing process. Gene array technology is having a major impact on the field of wound healing and has the potential to profoundly affect the way we understand the pathogenesis, diagnosis, prevention, and treatment of chronic wounds. Currently, gene array technology is used in the field of chronic wound healing to (1) understand the pathogenesis of pressure ulcers and venous ulcers, (2) understand the pathogenesis of diabetic foot ulcers, including the role that neuropathy may play in delayed healing of diabetic foot ulcers, and (3) determine the mechanism(s) of established and new local treatments, that is, pharmacogenomics for pressure ulcers and diabetic foot ulcers.

Cutaneous wound healing is a multistep process that requires the collaborative effort and precise coordination of many different tissues and cell types. These include epidermis, dermis, local vascular structures, and the immune system [1–3]. Analogous to these coordinated cellular efforts, successful wound healing requires also a dedicated, well-coordinated, clinical team.

On a cellular level, wound healing is a complex process that involves keratinocyte proliferation and migration, matrix deposition, vascular permeability, angiogenesis, and immune responses, and therefore depends on synchronized, multiple-signaling mechanisms. These signals include growth factors (eg, granulocyte/macrophage colony-stimulating factor, epidermal growth factor, vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived growth factor–BB, and keratinocyte growth factor–2), cytokines (eg, interleukins [IL]-1 and IL-8 and interferon-γ), hormones (eg, corticosteroids), and vitamins (eg, retinoids) [4–7]. Although cellular behavior during the wound-healing process is generally understood, the molecular mechanisms that coordinate them require more investigation.

Gene array technology is based on the selective specificity and affinity of complementary base pairing of the DNA and RNA molecules. The sequence information derived from the human genome project [8–11] has provided a new experimental tool for simultaneous readout of the cellular expression profile during the wound-healing process. As a complex, multistep process that involves different cell types and highly coordinated, interacting regulatory pathways, wound healing is an ideal biological process to be studied by gene array technology. Rather than focusing on individual genes, this technology allows us to further our understanding of wound healing by analyzing globally the functional pathways and interactions of cellular components that regulate, as well as carry out, cellular processes responsible for wound healing [12].

Gene array technology: molecular tool for gene-expression profiling in wounds

The use of gene arrays in research is based on the simple principle described by Southern [13] over a quarter of a century ago, which states that nucleic acids can be used to

* Corresponding author. Office tel.: +1-212-263-0748; laboratory tel.: +1-212-263-3988; fax: +1-212-263-8752.

E-mail address: tomicm01@med.nyu.edu

This work was supported in part by the United Spinal Association; the American Diabetes Association; Grants Nos. AR45974, NR08029, DK59424, and DK43093 from the National Institutes of Health; a Research Fellowship Award granted through the Dermatology Foundation; and a Rudolf Baer Foundation Research Grant.

0002-9610/04/$ – see front matter
doi:10.1016/S0002-9610(03)00293-9

interrogate nucleic acid molecules attached to a solid support [13]. This statement refers to placing immobilized complementary DNA (cDNA) probes on a surface, extracting the messenger RNA (mRNA) from tissues or cells and, after appropriate labeling, hybridizing the labeled mRNA to the surface. The amount of mRNA in the sample directly reflects the transcriptional activity of a gene, and the signal obtained upon hybridization is, therefore, a direct measurement of the particular gene activity. Comparison between different samples (eg, treated vs untreated; wounded vs unwounded or normal vs pathogenic) provides a transcriptional profile of the genes active during the treatment or any given process [14–16].

Perhaps the most notable capability of gene array technology is its ability to measure transcriptional activity (transcriptome) of a large number of genes simultaneously. This makes it possible to obtain information about the transcriptional activity of thousands of genes from a single hybridization experiment.

Gene arrays are divided into 2 groups: small- and large-scale gene arrays, based on the actual array number of analyzable genes. Small-scale arrays typically contain a few hundreds to several thousands of genes, whereas large-scale arrays may contain tens of thousands of cDNAs to be analyzed. Small-scale arrays are based on nylon membranes, which contain a variety of cDNA molecules either available commercially [17–19] or custom-made in the laboratory [20].

Recent advances in the technology of nonporous solid support surfaces for spotting DNA, as well as in photolithography, allow high-density synthesis of oligonucleotides and development of large-scale, gene array methodology [21,22]. Currently, 2 different methods of gene chip hybridization are available: cDNA and oligonucleotide arrays. cDNA arrays use a cDNA library as a probe source [15,23]. cDNA is amplified by polymerase chain reaction and spotted on glass slides. Alternatively, large oligonucleotides may be spotted instead of the amplified cDNA. Major advantages are an unlimited source of probes, as well as the simplification of custom design. Disadvantages of the cDNA array method are the requirement for significant investment in start-up and quality control, a much higher amount (tens of micrograms) of labeled RNA, and cross-hybridization among gene families. Oligonucleotide arrays use synthetic oligonucleotides derived from gene bank sequences, synthesized and chemically attached to the small surface [24]. Major advantages of the oligonucleotide array method are high reproducibility and smaller required amounts of RNA [25]. However, these chips are more expensive and not as easy to customize. The technology of microarrays is rapidly evolving. Many variations of the 2 basic large scale gene arrays are emerging, and novel approaches are being developed [26–28].

The 3 components of microarray analysis are sample preparation, array hybridization, and data mining/interpretation (Fig. 1). The source of the mRNA depends on experimental design and can originate either from cultured cells or tissue samples of specific tissue sections [29]. Sample preparation and array hybridization are essential steps, and the quality of information obtained depends on 3 factors: purity, amount, and veritable RNA labeling. The most involved of these factors is labeling. Numerous kits are available that make labeling, an inherently complex and delicate process, relatively straightforward. However, a common misunderstanding of array technology is that the experiment is almost complete when hybridization of the chip has been completed, and that this is a relatively "quick and dirty" way of obtaining a large amount of information about the process. On the contrary, the experiment begins only when the chip scanner generates an initial data file. After hybridization of the chips is completed and the data are collected, hundreds or thousands of genes are analyzed, depending on which type of array was used.

One of the essential components of data mining is validation of the gene list, that is, describing the genes and their functions [30]. This process includes evaluating various databases, extracting the information from gene banks and gene cards, identifying each gene and its function, and organizing these into functional clusters. Examples of these clusters include genes participating in apoptosis, DNA repair, and signaling cascade. When annotations are completed, the real analysis begins.

The first step is to construct a database or "data warehouse" [31]. This step is important because when several experiments are completed, there will be large data pools that can be analyzed simultaneously or used for comparative analyses. Therefore, to compare several different experiments, each experimental data group must be verified. It is also important that a single data type retain the same meaning throughout the database, regardless of the experimental data group [31]. As the quality of gene-expression data can vary among experiments, reliable statistical analyses are necessary for each expression measurement.

The true power of gene array analysis is derived from the analysis of multiple hybridizations to identify common patterns of gene expression. On the basis of our understanding of cellular processes, we infer that genes contained in the same pathway or responding to a common environmental challenge are coregulated and have similar patterns of expression.

Pattern recognition: making the identification

Gene array technology allows for the formation of a genetic (transcriptional) map of the normal wound-healing process, a crucial step in understanding the pathogenesis of chronic wounds. Simply put, to understand "what went wrong" in a nonhealing wound, one must understand what occurs during normal wound healing. Using large-scale gene arrays, a map of gene expression in normal wound healing is matched against that of each type of chronic (ie,

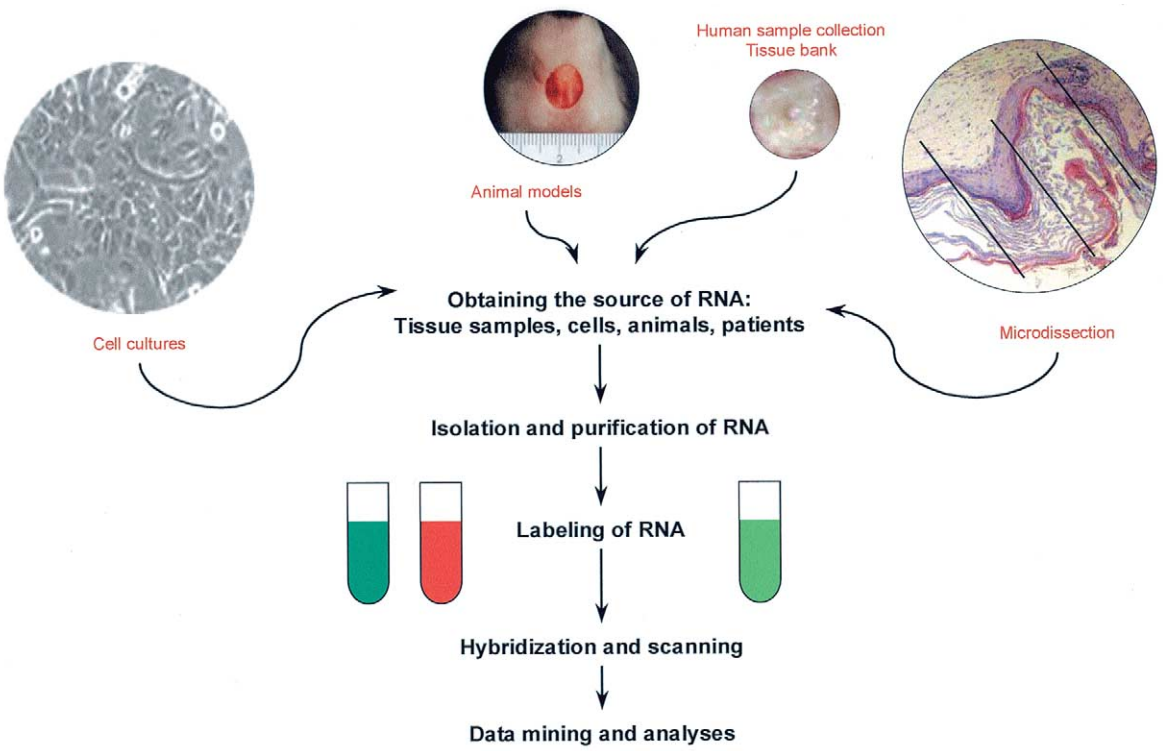

Fig. 1. Schematic representation of microarray analysis. Similar analysis is used to distinguish gene expression from cell culture, human wounds, and animal wounds.

nonhealing) wound. This matching generates a unique profile, or identification (ID), for each specific wound type (eg, venous reflux, diabetic foot, etc). This ID can then be used to locate specific malfunctioning cellular processes, rather than pinpointing only a few genes, and, in turn, to specify the most effective therapy or treatment modality for the particular wound.

Several reports in the literature describe the first attempts to find a genetic profile correlated with the repair of various tissues, such as skin, cornea, or spinal cord. All these studies have either used small-scale cDNA arrays, thus analyzing several thousand genes simultaneously [18,19,32,33] or focused on animal models [19,34,35]. Nevertheless, these pioneer publications represent a major step forward in understanding how various wounds heal. Interestingly, gene array analysis of early response of spinal cord injury shows similarity to the patterns displayed for skin reactions in the early stages of repair [36]. The use of gene array technology to understand pathogenesis of spinal cord injury has significant implications, because spinal cord injury patients have an extremely high incidence and prevalence of pressure ulcers. The information obtained from these comparative analyses will be used to identify specific categories of molecular defects. In addition, the gene chip analysis of the healing wound has revealed phenotypic similarities to squamous cell carcinoma [34].

In the near future, it will be possible to diagnose a particular subtype of chronic wound by using gene array technology and, therefore, choose the appropriate course of therapy. Pharmacogenomics, as an approach of high-throughput drug discovery, has already been developed and proved successful for a variety of diseases [37,38]. Ultimately, healthcare providers will have the ability to customize the therapy for each wound on each individual patient, rather than using ID to categorize and treat the wound.

Practical considerations

Choosing the sample

Although gene array analysis is extremely effective, several issues must be considered in regard to interpretable data [39]. The first is variability of the human samples collected from either normal or chronic wounds. The variability of normal skin is based on location of the body from which the sample is taken, as well as age, sex, and race of the donor, in addition to genetically derived variability. One of the major findings of the genome project is that human beings are not as diverse as previously thought to be the case [40–42]. Interestingly, initial analyses of normal human skin by using gene array technology have shown relatively small differences (approximately 1.7% of the genes analyzed are significantly different) among normal human skin samples of various donors [40–43].

Critical in gene chip analysis of a chronic wound is the understanding that various differing characteristics existing in different types of wounds but that are common to all

types is a physiologic impairment. The various types of chronic wounds include venous stasis ulcer, pressure ulcer, sickle cell ulcer, diabetic foot ulcer, and chronic wounds caused or exacerbated by radiation, chemotherapy, obesity, and infection. The individual wound type must be analyzed in terms of area, considering the (1) nonhealing edge, (2) wound base, or (3) adjacent skin that appears not to be involved as an immediate part of the wound bed, that is, the outer edge.

If a sample is taken from a particular area of the ulcer, because there is typically a need for multiple sample analyses, it is essential to obtain samples from the same area for each ulcer/patient. Otherwise, the data will not be interpretable. Physiologic variables also must be controlled (eg, bacteria or neuropathy). In addition, whether the samples are collected before or after debridement may greatly influence the results. It is important to understand that whichever conditions (ie, before or after debridement; with or without neuropathy, etc) and subject selection (particular age group of patients, specific underlying pathology, diabetic or nondiabetic, etc) one decides to use, the consistency in keeping the particular (preselected) variables constant is a necessity. Meaningful gene chip analysis of chronic wounds must consider all these variables and put maximum effort to maintain consistency in experimental design [44].

Evaluation and analysis of results

After the data are obtained, various methods of analysis are available, none of which is correct or incorrect, better or worse [45]. The various methods are different, and any single approach is not necessarily the sole basis for valid analysis. The common misconception in gene array analysis is that identical input should produce identical output, regardless of the analytical method. This, however, is not the case. Different methods view data differently, and the outcomes therefore also necessarily differ. By changing the analytical method, one is asking different questions and the answers cannot be identical.

To extract data quantitatively and qualitatively that are biologically significant, different analytical and computational methods must be used. Application of multiple analytical techniques allows for examination of different characteristics of the data. Various methods of clustering, such as hierarchical clustering, mutual information, and self-organized maps [46–52] are examples of the multiple analytical techniques needed. Clustering techniques are powerful and objective, and the algorithms are well defined and reproducible. In cluster analysis, each experiment represents a separate, distinct dimension in space, and the expression level measured for a given gene in that experiment represents its geometric coordinate [18]. All information about that gene can be represented by a point in expression-dimensional space. The more similar their patterns of expression, the closer the genes will appear in the diagram, and vice versa. Such visualization provides us with a means of measuring distances between the genes, and the clustering algorithms group genes together based on their distance in this space. In other words, this allows a translation of a multidimensional mathematical space in 2 dimensions.

Two considerations are important before clustering begins: (1) Should the data be adjusted in some way to emphasize relationships of similarity? (2) What measurement of distance should be used to group together related genes? The distance between any 2 genes is fundamental to placing them into groups, and finding clusters of similar genes depends on finding and grouping those close to each other [46,53]. The distance measure used, therefore, determines how close together similar genes will appear in any given place. If the measure used is too broad, it may be difficult to tell which genes are similar; if the measure is too detailed, it may be difficult to distinguish one similar gene from another.

From our perspective, any subjectivity in interpretation will most likely derive exclusively from selection of different algorithms and different normalizations. Therefore the challenge is to choose relevant data and to use the algorithms such that the gene-expression classification that emerges obtains the data in a rational and biologically meaningful manner. This is a time-consuming process. Data analysis and evaluation easily requires up to 80% to 90% of the total time in microarray experiments. Not only is computer analysis itself relatively slow for such large data sets, but also evaluation of the results obtained requires significant time and effort. Additionally, new, more sophisticated computational techniques are being developed rapidly, which has the effect of extending the time necessary for completion of the analysis. Newer methods generally provide new capabilities for analysis rather than replacing the existing ones.

Confirmation of the results must always be addressed. Evaluation of the results for all genes analyzed is not possible, but evaluations using standard molecular and cellular biology techniques, such as Northern blots or real-time polymerase chain reaction, Western blots, as well as immunocytochemistry and in situ hybridizations, are necessary. The choice as to which genes should be evaluated is limited by the availability of appropriate antibodies or probes, but it is critical to select several genes from a strongly regulated group, both induced and repressed (eg, 5-fold or more), and also from a group mildly regulated (between 2.5- and 5-fold). Furthermore, a new gene array experiment featuring the effects of a given molecule also can be used to evaluate initial results [54].

The meaning of the data

The most difficult task in these analyses is to put the gathered information in a biological context in order to interpret the real meaning of the data. Unfortunately, few publications provide these insights (see, for example, Li et al [55]); most provide “tablecloths” of data that contribute

to overall skepticism about usefulness of the gene arrays rather than meaningful biological explanations. Our position is that data gathered using the various cluster-analysis methods described above should result in a solid basis for meaningful biological interpretation.

Future directions

When obtained, the chronic wound IDs will be used to select the appropriate targets for pharmaceutical intervention. However, gene array technology also should be used for evaluation of current treatment modalities [56]. For example, a considerable amount of literature is available that explains why a particular growth factor therapy did not have therapeutic success. Different opinions are offered in the discussions of such papers, but gene array technology will provide us with comprehensive answers. Furthermore, clinical trials have shown that tissue therapy is more successful, but questions remain as to its basis and mechanism of action. Gene arrays will provide us with answers to the issue of success of particular therapeutic approaches.

Not all changes that may cause chronic wounds are based on transcriptional effects. A gene product may be regulated in different ways, either by posttranscriptional or posttranslational modifications. In the near future, one can expect synergy between genomics and proteomics [57]. This merger should provide complete profiles of the pathology of chronic wounds, that is, molecular defects that lead to inhibition of the wound-healing process.

Acknowledgments

Special thanks to Dr. Miki Blumenberg for generous support, helpful criticisms, and mostly for such inspirational belief and extensive knowledge in gene chip technology, which he shared generously. We are grateful to Hyacinth Entero and Tom Jacobs for generous help in preparation of this article. We also thank Dr. Irwin M. Freedberg for support and exceptional dedication to our work.

References

[1] Yamaguchi Y, Yoshikawa K. Cutaneous wound healing: an update. J Dermatol 2001;28:521–534.
[2] Sherratt JA, Dallon JC. Theoretical models of wound healing: past successes and future challenges. C R Biol 2002;325:557–564.
[3] Ruszczak Z, Schwartz RA. Modern aspects of wound healing: an update. Dermatol Surg 2000;26:219–229.
[4] Woolley K, Martin P. Conserved mechanisms of repair: from damaged single cells to wounds in multicellular tissues. Bioessays 2000; 22:911–919.
[5] Tomic-Canic M, Komine M, Freedberg IM, Blumenberg M. Epidermal signal transduction and transcription factor activation in activated keratinocytes. J Dermatol Sci 1998;17:167–181.
[6] Mansbridge JN, Liu K, Pinney RE, Patch R, Ratcliffe A, Naughton GK. Growth factors secreted by fibroblasts: role in healing diabetic foot ulcers. Diabetes Obes Metab 1999;1:265–279.
[7] Martin P. Wound healing—aiming for perfect skin regeneration. Science 1997;276:75–81.
[8] McPherson JD, Marra M, Hillier L, et al. A physical map of the human genome. Nature 2001;409:934–941.
[9] Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. Science 2001;291:1304–1351.
[10] Collins FS, Green ED, Guttmacher AE, Guyer MS. A vision for the future of genomics research. Nature 2003;422:835–847.
[11] Collins FS, Morgan M, Patrinos A. The Human Genome Project: lessons from large-scale biology. Science 2003;300:286–290.
[12] Cole J, Isik F. Human genomics and microarrays: implications for the plastic surgeon. Plast Reconstr Surg 2002;110:849–858.
[13] Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503–517.
[14] Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. Nat Genet 1999;21:33–37.
[15] Iyer VR, Eisen MB, Ross DT, et al. The transcriptional program in the response of human fibroblasts to serum. Science 1999;283:83–87.
[16] Copland JA, Davies PJ, Shipley GL, Wood CG, Luxon BA, Urban RJ. The use of DNA microarrays to assess clinical samples: the transition from bedside to bench to bedside. Recent Prog Horm Res 2003;58:25–53.
[17] Fletcher ST, Baker VA, Fentem JH, Basketter DA, Kelsell DP. Gene expression analysis of EpiDerm following exposure to SLS using cDNA microarrays. Toxicol In Vitro 2001;15:393–398.
[18] Varela JC, Goldstein MH, Baker HV, Schultz GS. Microarray analysis of gene expression patterns during healing of rat corneas after excimer laser photorefractive keratectomy. Invest Ophthalmol Vis Sci 2002;43:1772–1782.
[19] Cao Z, Wu HK, Bruce A, Wollenberg K, Panjwani N. Detection of differentially expressed genes in healing mouse corneas, using cDNA microarrays. Invest Ophthalmol Vis Sci 2002;43:2897–2904.
[20] Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. Curr Biol 1999;9:939–945.
[21] Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467–470.
[22] Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D. Light-directed, spatially addressable parallel chemical synthesis. Science 1991;251:767–773.
[23] Duggan DJ, Bittner M, Chen Y, Meltzer P, Trent JM. Expression profiling using cDNA microarrays. Nat Genet 1999;21:10–14.
[24] Lipshutz RJ, Fodor SP, Gingeras TR, Lockhart DJ. High density synthetic oligonucleotide arrays. Nat Genet 1999;21:20–24.
[25] Rogojina AT, Orr WE, Song BK, Geisert EE Jr. Comparing the use of Affymetrix to spotted oligonucleotide microarrays using two retinal pigment epithelium cell lines. Mol Vis 2003;9:482–496.
[26] Harris TM, Massimi A, Childs G. Injecting new ideas into microarray printing. Nat Biotechnol 2000;18:384–385.
[27] Okamoto T, Suzuki T, Yamamoto N. Microarray fabrication with covalent attachment of DNA using bubble jet technology. Nat Biotechnol 2000;18:438–441.
[28] Leiva IM, Emmert-Buck MR, Gillespie JW. Handling of clinical tissue specimens for molecular profiling studies. Curr Issues Mol Biol 2003;5:27–35.
[29] Bowtell DD. Options available—from start to finish—for obtaining expression data by microarray. Nat Genet 1999;21:25–32.
[30] Zhong S, Li C, Wong WH. ChipInfo: software for extracting gene annotation and gene ontology information for microarray analysis. Nucleic Acids Res 2003;31:3483–3486.
[31] Bassett DE Jr, Eisen MB, Boguski MS. Gene expression informatics—it's all in your mind. Nat Genet 1999;21:51–55.
[32] Cole J, Tsou R, Wallace K, Gibran N, Isik F. Early gene expression profile of human skin to injury using high-density cDNA microarrays. Wound Repair Regen 2001;9:360–370.

[33] Pedersen TX, Leethanakul C, Patel V, et al. Laser capture microdissection-based in vivo genomic profiling of wound keratinocytes identifies similarities and differences to squamous cell carcinoma. Oncogene 2003;22:3964–3976.
[34] Li X, Mohan S, Gu W, Baylink DJ. Analysis of gene expression in the wound repair/regeneration process. Mamm Genome 2001;12:52–59.
[35] Spies M, Dasu MR, Svrakic N, et al. Gene expression analysis in burn wounds of rats. Am J Physiol Regul Integr Comp Physiol 2002;283: R918–R930.
[36] Song G, Cechvala C, Resnick DK, Dempsey RJ, Rao VL. GeneChip analysis after acute spinal cord injury in rat. J Neurochem 2001;79: 804–815.
[37] Howbrook DN, van der Valk AM, O'Shaughnessy MC, Sarker DK, Baker SC, Lloyd AW. Developments in microarray technologies. Drug Discov Today 2003;8:642–651.
[38] Sax JK, El-Deiry WS. p53-induced gene expression analysis. Methods Mol Biol 2003;234:65–71.
[39] Dobbin K, Shih JH, Simon R. Questions and answers on design of dual-label microarrays for identifying differentially expressed genes. J Natl Cancer Inst 2003;95:1362–1369.
[40] Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860–921.
[41] Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 2001;409:928–933.
[42] Gazel A, Ramphal P, Rosdy M, et al. Transcriptional profiling of epidermal keratinocytes: comparison of genes expressed in skin, cultured keratinocytes and reconstructed epidermis, using large DNA microarrays. J Invest Dermatol 2003;121:1459–1468.
[43] Cole J, Tsou R, Wallace K, Gibran N, Isik F. Comparison of normal human skin gene expression using cDNA microarrays. Wound Repair Regen 2001;9:77–85.
[44] Huang S, Qian HR, Geringer C, Love C, Gelbert L, Bemis K. Assessing the variability in GeneChip data. Am J Pharmacogenomics 2003;3:279–290.
[45] Detours V, Dumont JE, Bersini H, Maenhaut C. Integration and cross-validation of high-throughput gene expression data: comparing heterogeneous data sets. FEBS Lett 2003;546:98–102.
[46] Quackenbush J. Computational analysis of microarray data. Nat Rev Genet 2001;2:418–427.
[47] Wen X, Fuhrman S, Michaels GS, et al. Large-scale temporal gene expression mapping of central nervous system development. Proc Natl Acad Sci U S A 1998;95:334–339.
[48] Michaels GS, Carr DB, Askenazi M, Fuhrman S, Wen X, Somogyi R. Cluster analysis and data visualization of large-scale gene expression data. Pac Symp Biocomput 1998:42–53.
[49] 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;95:14863–14868.
[50] Weinstein JN, Myers TG, O'Connor PM, et al. An information-intensive approach to the molecular pharmacology of cancer. Science 1997;275:343–349.
[51] Tamayo P, Slonim D, Mesirov J, et al. Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. Proc Natl Acad Sci U S A 1999;96: 2907–2912.
[52] Tavazoie S, Hughes JD, Campbell MJ, Cho RJ, Church GM. Systematic determination of genetic network architecture. Nat Genet 1999;22:281–285.
[53] Heyer LJ, Kruglyak S, Yooseph S. Exploring expression data: identification and analysis of coexpressed genes. Genome Res 1999;9: 1106–1115.
[54] Chen CC, Chen N, Lau LF. The angiogenic factors Cyr61 and connective tissue growth factor induce adhesive signaling in primary human skin fibroblasts. J Biol Chem 2001;276:10443–10452.
[55] 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: 2533–2535.
[56] Kramer JA, Kolaja KL. Toxicogenomics: an opportunity to optimise drug development and safety evaluation. Expert Opin Drug Saf 2002; 1:275–286.
[57] Knezevic V, Leethanakul C, Bichsel VE, et al. Proteomic profiling of the cancer microenvironment by antibody arrays. Proteomics 2001; 1:1271–1278.