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DNA Microarray Technology in Dermatology

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In recent years, DNA microarray technology has been used for the analysis of gene expression patterns in a variety of skin diseases, including malignant melanoma, psoriasis, lupus erythematosus, and systemic sclerosis. Many of the studies described herein confirmed earlier results on individual genes or functional groups of genes. However, a plethora of new candidate genes, gene patterns, and regulatory pathways have been identified. Major progresses were reached by the identification of a prognostic gene pattern in malignant melanoma, an immune signaling cluster in psoriasis, and a so-called interferon signature in systemic lupus erythematosus. In future, interference with genes or regulatory pathways with the use of different RNA interference technologies or targeted therapy may not only underscore the functional significance of microarray data but also may open interesting therapeutic perspectives. Large-scale gene expression analyses may also help to design more individualized treatment approaches of cutaneous diseases.
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Gene microarray technology started in the early nineties of the last century. It was demonstrated that peptides may be synthesized on small silicon chips by photolithographic synthesis.[1,2] This technique was then applied to short DNA fragments, generating so-called DNA microarrays (unless otherwise stated, in the present review the term microarrays refers to DNA microarrays). By use of these microarrays, the amount mRNA molecules in a given biological sample may be quantified with high accuracy via complementary binding of mRNAs to the DNA probes fixed on the microarray.[3] The development of oligonucleotide DNA microarrays was paralleled by that of cDNA microarrays, using 600 to 2000 bases cDNA molecules as probes.[4] Recent progress in array technology demonstrated equal sensitivity for DNA microarrays carrying probes of 60 to 80 bases in length. At present, the latest oligonucleotide and cDNA microarrays carry probes for expression analysis of all currently known genes (more than 35,000). In parallel to these whole-genome chips, several companies offer more specific microarrays for mRNA expression analysis of specific gene subsets.

In the mentioned report by Lockhart and coworkers,[3] it was demonstrated that oligonucleotide DNA microarrays could be used to measure mRNA molecules within a wide linear range of 3 to 4 orders of magnitude, with a sensitivity of a few molecules per cell. Indeed, later studies confirmed that the detection lower limit of current microarray technology appears to be around ten copies of mRNA per cell.[5,6] As a consequence, low abundance genes such as transcription factors may sometimes be lost, or at least not reliably be detected by DNA microarrays. When comparing results from different technical platforms, consistency of data for differentially expressed genes was disappointing, as reported a few years ago.[7] This was in part attributable to the fact that in these analyses low abundance genes, which may often not accurately be detected, were not filtered out. Moreover, sufficient probe sequence information was not available of different platforms, and different probe sequences for individual genes could thus not be taken into consideration. As reported recently by the Microarray Quality Control Project, high intra- and interplatform consistency may be reached due to an optimization of probe sequences and appropriate filtering.[8]

High specificity of DNA microarrays allows detection of the exchange of even one single base when using appropriate short oligonucleotides (so called single-base resolution). As a consequence, oligonucleotide DNA microarrays also may be used for DNA sequencing.[9] Specific DNA microarrays were used to detect mutations in certain tumor-associated genes such as *BRCA1* and *p53*, respectively.[10,11] A further application of DNA microarray technology, cur-

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Table 1 Current Key Genomics and Proteomics Technologies

Technology	Targets	Number of Targets	Sensitivity*	Sensitivity Threshold†	Specificity	References
Oligonucleotide and cDNA microarrays	mRNAs	>35,000 (whole genome)	High (>90%)	Low (~10 mRNA copies per cell)	High (70-90%)‡	5,6,12
Oligonucleotide microarrays	SNPs	~ 1,000,000	High (>95%)	NA	High (>95%)	14-16
Protein and antibody microarrays	Proteins	1,000-5,000	High (>90%)	Low ~ 20 pg	Low to intermediate (20-50%)	18,19
2D gel electrophoresis combined with mass spectrometry	Proteins	100,000-500,000	Low (<10%)	Low ~ 1 ng	High (>75%)	18,19

NA, not applicable.

*Sensitivity refers to the number of mRNAs, SNPs, or proteins detected in a complex background relative to the total number of mRNAs, SNPs, or proteins that might theoretically be detected by this particular technology.

†Sensitivity threshold refers to the lower limit of sensitivity for a specific mRNA or protein.

‡Estimated value based on analyses of a limited number of genes. Systematic studies on probe specificity for all genes are lacking.

rently attracting widespread attention, is the identification of single nucleotide polymorphisms (SNPs).[12-17] SNPs are homozygous or heterozygous nucleotide variations in the human genome, with an estimated incidence of about one SNP every 300 to 1000 base pairs (bps). SNPs may contribute to tumor development and progression and may predispose one to a variety of different diseases, such as diabetes, high blood pressure, and arthritis.[17] At present, the total number of SNPs in public databases exceeds 9 million. Current DNA microarray technology may detect up to 1 million human SNPs.[16,17] Oligonucleotide microarrays used for SNP detection differ from those for mRNA expression analysis. In principle, in SNP arrays four oligonucleotide probes are designed to interrogate a single position. One probe binds with perfect complementarity to the reference sequence in the sample DNA. The other 3 differ from the first at the interrogation position by substitution of 1 of the 3 other bases, which leads to nonperfect binding.[12] The complementary probe variant results in significantly enhanced signal intensity compared with the others, thus allowing exact identification of a particular SNP.

Because differential gene expression does not necessarily translate into differential protein expression, technological platforms for large-scale protein (proteome) analyses have been developed in recent years.[18] In so-called forward-phase protein microarrays, predefined antibodies are immobilized on a glass slide to interrogate a given protein sample (eg, a cellular lysate).[18,19] In reverse-phase microarrays, a complex protein mixture is immobilized on a glass slide, which is then probed with specific antibodies.[18,19] The detection lower limit of protein concentrations when one is using protein microarrays may reach a 10-cell equivalent. However, the most commonly used technology for proteome analysis is a combination of 2D gel electrophoresis for protein separation and mass spectrometry for protein identification.[18] Theoretically, this technology allows the identification of any protein in a given sample, in contrast to the technological limitations of mentioned antibody or protein arrays. Current mass spectrometry may be performed with high mass accuracy (<10 parts per million). However, even with high-resolution protein separation of 2D gels, the number of proteins that may be identified is generally less than 10,000. A summary of technological issues of key genome and proteome technologies is given in Table 1.

A major challenge in particular for DNA microarray analyses is data processing and biostatistics. Before microarray data may be subjected to detailed analysis, preprocessing of raw data must be performed,[20,21] including image analysis, summarization and normalization.[22] In particular, each microarray must be normalized to all other microarrays of an experiment so that all microarrays are comparable.[23] Statistical analysis of microarray data includes so-called supervised and unsupervised methods. Supervised methods generally are applied when a class label for each sample is known, for instance, each sample may unambiguously be attributed to a defined clinical or histopathological entity. Supervised clustering methods may then identify differentially expressed genes or predict the class label of new unknown samples. The corresponding computational techniques are support vector machines, neural networks, or partitioning around medoids (ie. PAM) approaches.[24-26] These approaches normally use a majority of samples as a training set, on which a so-called classifier is build. This classifier can then be used to predict the classification of a test sample.

If there are no clearly defined groups or subgroups with class labels, unsupervised methods (clustering) may be applied. A series of different methods are in use for cluster analysis, like *k-means* clustering or hierarchical clustering, as described by Eisen and coworkers.[27] In the latter case, hierarchical cluster trees are generated that juxtapose genes based on the similarity of expression profiles. For better optical presentation, expression levels of genes are represented by color squares. Clustering may also be performed by so-called self-organizing maps (SOMs),[28] which may particularly be useful when analyzing time course experiments. Taken together, tight collaboration between clinicians, molecular biologists and mathematicians is often indispensable for optimal design and utilization of microarray experiments.

Table 2 DNA Microarray Studies on Skin Cancer

Tumor Type	Gene Expression in Tumorigenic or Metastatic Phenotype or Bad Prognosis Group	Cell Type, Tissue	Array Type	References
Malignant melanoma	RhoC ↑, fibronectin ↑, collagen α2(I) ↑, collagen α1(III) ↑, matrix Gla protein ↑, fibromodulin ↑, biglycan ↑, thymosin β4 ↑	Cell lines A375P, A375M1, A375M2, A375SM	HUM 6.8K microarray, Affymetrix, 7,000 probe sets	34
	WNT5 ↑, fibronectin ↑, syndecan 4 ↑, tropomyosin ↑, MART1 ↓, CD63 ↓, PGAM1 ↓	Primary melanomas, melanoma metastases, cell lines	cDNA microarray, 8,000 probes	36-38
	Osteopontin ↑, CXCL1 ↑, RAB32 ↑, CKS2 ↑, CENPF ↑, CXCL10 ↑, DSC3 ↓, DST ↓, WIF1 ↓	Benign nevi, primary melanomas, melanoma metastases	cDNA microarray, Research Genetics, 21,000 probes	40
	MCM3, 4, 6 ↑, geminin ↑, Cdc2 ↑, CKS2 ↑, CCNB1 ↑, Cdc6 ↑, KIF2C ↑, STK6 ↑, CENPF ↑, PCNA ↑, PROM2 ↓, SPINT2 ↓, CST3 ↓, CST5 ↓, CXCL14 ↓	Primary melanomas (differing by distant metastasis-free survival)	Whole-human-genome 44K microarray, Agilent, 44,000 probes	41
Basal cell carcinoma	PTCH 1 ↑, gli2 ↑, WNT5a ↑, Frizzled D2 ↑, D7 ↑, D8 ↑, basonuclin 2 ↑, chromogranin A ↑, CTNNBIP1 ↓, TCF-4 ↓	Normal skin, basal cell carcinomas	U133 Plus 2.0 microarray, Affymetrix, 55,000 probe sets	45
Squamous cell carcinoma	E-cadherin ↑, MAP4K4 ↑, N-Myc ↑, N-Myc and STAT interactor (NMI) ↑, Rab31 ↑, MMP-1 ↑, MMP-9 ↑, CGI-39 ↓, ERCC1 ↓	Normal skin, squamous cell carcinomas	U133A microarray, Affymetrix, 22,000 probe sets	47

Arrows indicate upregulated (↑) or downregulated (↓) gene expression.

Malignant Melanoma

From the very beginning, DNA microarrays have been used to identify pathogenic genes or gene patterns in tumors.[29,30] Some of the recent studies were even able to identify patterns associated with clinical outcome.[31,32] In the first microarray study on malignant melanoma, gene expression profiles of 2 melanoma cell lines, UACC-903 and UACC-903 (+6), were compared, using a 1161-item cDNA microarray.[33] In the UACC-903 (+6) cell line, introduction of a normal chromosome 6 reversed the tumorigenic phenotype of the UACC-903 parental cell line. The chemokine monocyte chemoattractant protein 1 (MCP-1), which was one of the genes with the most significant differences, showed down-regulation in the tumorigenic phenotype. It was demonstrated in later experiments that low levels of MCP-1 in melanoma tissues indeed supported melanoma growth. Melanoma antigen tyrosinase-related protein 1 (TRP 1/gp 75) showed enhanced expression in the tumorigenic phenotype, a finding in line with earlier observations, showing that TRP 1/gp 75 is a marker for early malignant transformation of melanoma cells.

Table 2 summarizes results of more recent microarray studies on malignant melanoma, basal cell, and squamous cell carcinoma. Clark and coworkers analyzed gene expression profiles of nonmetastatic and metastatic melanoma cells with the same genetic background.[34] An outstanding role for melanoma metastasis was proposed for RhoC, a member of the Rho family of GTPases. Indeed, in an experimental metastasis model overexpression of wild-type RhoC in nonmetastasizing melanoma cells resulted in enhanced metastasis. Overall, genes encoding for cytoskeletal and extracellular matrix proteins, such as fibronectin, different types of collagen, matrix Gla protein, fibromodulin, biglycan, and thymosin β4, appeared to play an important role in melanoma metastasis. In line with this, in a first series of microarray experiments we identified the 2 extracellular matrix molecules thrombospondin 2 and desmoglein 2 as being differentially expressed in melanoma cell lines of different metastatic behavior.[35]

The first study that provided evidence for the existence of gene patterns of prognostic significance in malignant melanoma was conducted by Bittner an coworkers.[36] Expression of 7000 genes was analyzed in 31 melanoma specimens with the use of cDNA microarrays. Hierarchical clustering of data identified a major cluster of 19 melanoma specimens. Melanoma cells derived from these tissues showed reduced motility, invasive ability, and vasculogenic mimicry. Gene expression profiles of these melanomas might thus represent less-aggressive behavior of tumor cells. Indeed, authors found a tendency for a better prognosis of patients belonging to this major cluster, yet not reaching statistical significance. Within this cluster, reduced expression was described for integrin β1, integrin β3, syndecans, and vinculin. In line with Clark and coworkers, specimens outside this cluster showed enhanced expression of fibronectin.[34] Expression levels of WNT5, which was also a good discriminator between the 2 clusters, correlated with increased motility and invasiveness of melanoma cells.[37] Moreover, high WNT5 expression in melanoma tissues was associated with worse prognosis of affected patients.[38]

In a recent study using a cDNA microarray with 4467 probes, gene profiles of 22 melanocytic tumors, including benign nevi, primary melanomas, and melanoma metastases, were analyzed.[39] Laser-capture microdissection was performed to specifically address gene profiles of melanocytes or melanoma cells. Four highly differentially expressed genes allowed correct classification of different tumor stages with

82% accuracy. Among these were genes encoding for tyrosinase related protein 2, translation initiation factor 2γ, and ubiquitin conjugating enzyme E2I.

Gene expression at different stages of malignant melanoma development was analyzed in a further more comprehensive study with the use of a cDNA microarray with more than 20,000 probes.[40] First, the authors compared gene expression profiles of vertical and horizontal growth phase melanoma cells in one primary melanoma. Surprisingly, vertical growth phase melanoma cells showed down-regulated genes only. Among these were genes in functional categories of cell adhesion and extracellular matrix organization, such as desmocollin 2, matrix metalloproteinase 10, cadherin 3, and integrin α2. The different gene patterns identified in vertical or radial growth phase were able to differentiate between 2 subtypes of metastases. Evidence was provided that the different molecular patterns in metastases might be of prognostic relevance, because all 5 patients within group one had died, whereas 4 of 12 patients in group 2 were still alive without evidence of disease. Genes that showed up-regulation in primary melanomas compared with benign nevi included osteopontin, CXCL1, and RAB32, all known to play a role in melanoma progression.

Overall, 83 tumors were included in a study on prognostic gene patterns in primary melanomas in which researchers used a whole-genome oligonucleotide microarray.[41] Of the initial 83 specimens, 58 with at least 4 years follow-up information were included in further analyses. A classifier of 254 genes was able to differentiate between groups of 4-year distant metastasis free survival and distant metastasis within this period. Molecules with enhanced expression in the bad prognosis group belonged to the functional groups of cell cycle regulation, mitosis, and DNA replication, such as minichromosome maintenance genes 3, 4, and 6 (MCM3, 4, 6), geminin, Cdc2, CKS2, CCNB1, Cdc6, KIF2C, STK6, CENPF, and PCNA. Interestingly, the 254-gene classifier was an independent prognostic factor with an accuracy rate similar to well-known factors like tumor thickness and ulceration.

Because tissue hypoxia is a well-known tumor progression factor, our group analyzed gene expression patterns of hypoxic melanoma cells.[42] Within one gene cluster, ephrin-A1 and Cyr61 were identified as 2 putative, hypoxia-inducible angiogenesis factors for malignant melanoma. Ephrin-A1 had been described earlier by an independent group as a marker for tumor progression in malignant melanoma.[43] Ephrin-A2 was identified as a progression marker for malignant melanoma in the study of Bittner and coworkers.[36]

Laser-capture microdissection of primary melanomas and metastases combined with microarray technology was used by our group to identify tumor progression and metastasis-related genes.[44] In accordance with the findings of Winnepenninckx and coworkers,[41] gene patterns associated with tumor thickness and metastasis, respectively, were identified. Enhanced gene expression was found for genes belonging to gene ontology categories of cell cycle regulation and mitosis, including CKS2, Cdc6, KIF2C, and STK6. Metastasis was associated with reduced expression of cell adhesion molecules, such as E-cadherin and desmocollins 1 and 3.

Basal and Squamous Cell Carcinoma

Little is known about gene patterns in basal and squamous cell carcinoma. One recent study used a whole-genome microarray with more than 50,000 probe sets to analyze 20 basal cell carcinomas compared with normal control skin.[45] Overall, 3921 genes were differentially expressed. Up-regulation was observed in basal cell carcinomas for genes involved in hedgehog and Wnt signaling, such as patched homologue 1, gli2, WNT5a, and frizzled D2, D7 and D8, supporting current concepts of basal cell carcinoma development.[46] Chromogranin A, a molecule associated with neuroendocrine differentiation, showed most significant differences and was suggested as a marker for basal cell carcinoma. Although its role for basal cell carcinoma remains to be determined, a series of other tumors, such as nonsmall cell lung carcinoma and prostate cancer, also show enhanced expression of this molecule.

A study on differentially expressed genes in squamous cell carcinomas and actinic keratoses compared with normal skin identified 118 candidates out of more than 22,000 genes tested.[47] All cancerous tissues stemmed from immunosuppressed patients, normal tissues stemmed from both immunosuppressed and immunocompetent patients. Many of the up-regulated genes in carcinomas and actinic keratoses, such as E-cadherin, MAP4K4, N-Myc, and N-Myc and STAT interactor (NMI), Rab31, MMP-1, and MMP-9 have been described to play a role in other cancer types. Interestingly, there were not significant differences in gene patterns between squamous cell carcinomas and actinic keratoses.

Psoriasis

Psoriasis is a polygenic chronic inflammatory skin disease, where epidermal keratinocytes and inflammatory T-cells are of central pathogenic importance.[48] Psoriasis may even be regarded as an autoimmune disease.[49,50] Table 3 summarizes results of recent microarray studies on psoriasis and other inflammatory skin diseases. One earlier study compared gene expression patterns of lesional skin from 15 psoriasis patients with patterns of normal skin from the same patients and normal controls with a microarray with 12,000 gene probes.[51] The authors used *k-means* clustering to identify significant differences between diseased and normal skin. Overall, 177 differentially expressed genes were identified. Most of these showed up-regulation in diseased skin, such as S100 family members S100A2 and S100A7 to 9, a finding that was confirmed by 2 other microarray studies.[52,53] Moreover, well-known psoriasis-related genes, such as β-defensin 2, interleukin-8, and CD68, were significantly up-regulated in diseased skin. The biological relevance of these findings was underscored by the fact that many genes identified in this study mapped to known psoriasis susceptibility loci (eg, 1q21, 3q21, and 14q31-32).

Shortly thereafter, this latter study was extended by use of a new microarray type with 63,100 oligonucleotide probes,

Table 3 DNA Microarray Studies on Inflammatory Skin Diseases

Disease	Gene Expression in Affected Skin	Tissue	Array type	References
Psoriasis	S100A2 ↑, S100A7-A9 ↑, IL-8 ↑, DEFB2 ↑, CD68 ↑, CD47 ↑, ECGF1 ↑, ANXA1 ↑, KRT15 ↓, MT1L ↓	Normal, uninvolved and involved skin	U95A microarray, Affymetrix, 12,000 probe sets	51
	S100A7 ↑, S100A9 ↑, S100A12 ↑, FABP5 ↑, DEFB2 ↑, MMP12 ↑, CD47 ↑, STAT1 ↑, TNXA ↓, TIMP3 ↓	Uninvolved and involved skin	HuGeneFL microarray, Affymetrix, 7,000 probe sets	52
	S100A7-A9 ↑, IL-8 ↑, ECGF1 ↑, PBEF ↑, STAT1 ↑, SCYA2 ↑, SCYA19 ↑, SCYA21 ↑, SDF ↑, CDKN1C ↓, SCYA27 ↓, ITGB1 ↓	Normal, uninvolved and involved skin	U95A-E microarrays, Affymetrix, 63,000 probe sets	54
SLE	OASL ↑, LY6E ↑, MX1 ↑, PRKR ↑, ICAM1 ↑, SCYA3 ↑, XIAPAF1 ↑, LCK ↓, TCR β ↓, CD1C ↓	PBMC of control and SLE patients	U95A microarray, Affymetrix	64,71
	TRIP14 ↑, OAS1 ↑, TAP1 ↑, TRAIL ↑, MX1 ↑, MX2 ↑, XIAPAF1 ↑, IFIT4 ↑, MCP-1 ↑, DC-LAMP ↑, TCR δ ↓, DAP3 ↓	PBMC of control and SLE patients	U95A microarray, Affymetrix	74
	IFN-ω ↑, IFIT1 ↑, IFIT2 ↑, IFIT4 ↑, OAS1 ↑, OAS2 ↑, OASL ↑, LY6E ↑, TCRα ↓, TCRδ ↓	PBMC of control and SLE patients	U95A microarray, Affymetrix	72
SSc	CALR ↑, COL15A1 ↑, NID2 ↑, CTGF ↑, FKBP1A ↑, CDH5 ↑, THY1 ↑, CD53 ↑, IGHG3 ↑, BMP10 ↓, WIF-1 ↓	Normal, uninvolved and involved skin	U95A microarray, Affymetrix	84
	COLA7 ↑, COLA18 ↑, CD44 ↑, MT1A ↑, MT1B ↑, DSP ↑, VEGFB ↓, SGK ↓	Fibroblast cultures of control and SSc patients	Spotted oligonucleotide microarray, 16,600 probes	85

Arrows indicate upregulated (↑) or downregulated (↓) gene expression.

which covers all known genes, including expressed sequence tags.[54] The number of differentially expressed genes increased to 1338. Authors identified a major cluster of 131 immune signaling genes. In this cluster, overall 19 chemokines were identified, some of which have already been described in psoriasis, such as interleukin (IL)-8 and Gro-α. However, many were indeed new, such as small inducible cytokines SCYA19, SCYA21, and SDF (stromal cell-derived factor). Overlapping binding motifs for transcription factors were identified in upstream promoter elements of these immune-regulatory genes. These included motifs for transcription factors c-Ets-2, NFκB, AP-1, and IRF2-ISRE (interferon response factor 2–interferon-stimulated response element), regulating S100A7-A9, KRT6A, LDLR, LAMP3, HBP17 (heparin binding protein); OAS1, OAS2 (2,5-oligoadenylate synthetase 1 and 2); and ISG15 (interferon stimulated protein).

Our group analyzed gene expression profiles of peripheral blood mononuclear cells (PBMCs) from psoriasis patients with severe generalized disease before and after classical dithranol treatment.[55] Data were confirmed by real-time RT-PCR in an independent set of patients. Up-regulation in the diseased stage was found for interleukin-8, COX-2, PBEF, ANXAIII, TNFAIP6, and S100P. CDKN1C, also termed p57Kip2, was the only gene that showed significant up-regulation in the cured stage. Interestingly, CDKN1C acts as a cell cycle inhibitor in T cells.[56] It was demonstrated by use of support vector machines that a combination of both IL-8 and CDKN1C was able to differentiate between the 2 disease stages with a high prediction accuracy. These findings are highly suggestive for an interaction between both genes. Gene expression profiles of PBMCs from psoriasis patients receiving oral pimecrolimus treatment were analyzed in another study.[57] This treatment modality led to 80% clearing of skin lesions after 4 weeks. Of a total of 7129 genes tested, significant down-regulation was observed for molecules involved in antigen presentation, chemotaxis and leukocyte migration, and inflammation, including HLA-E, HLA-F, L-selectin, LFA1, RANTES, and prostaglandin endoperoxide synthase 1. Oestreicher and coworkers tested the effects of treatment with interleukin-11 or Cyclosporin A on gene expression patterns in psoriatic skin.[52] Response rates to treatment reached from 60% and 90%. By use of SOMs 159 differentially expressed genes were divided into 4 different clusters. Two clusters contained down-regulated genes under treatment. Among these were S100A9, S100A12, ID4, KRT16, monocyte chemotactic protein 1 (SCYA2), HBP17, and CCNF. Some of these were down-regulated by treatment with cyclosporin A but not with rhIL-11, for instance, HBP17 and CCNF. Taken together, the latter 3 studies showed that gene expression profiles might be useful for treatment monitoring or might even be used to predict treatment responses.

Reischl and coworkers identified WNT5a as an interesting candidate molecule for psoriasis.[58] Their findings of 179 differentially expressed genes in psoriatic compared with normal skin largely confirmed data from earlier studies.[54] However, increased expression of WNT5a in psoriatic plaques compared with uninvolved skin from the same patients was described for the first time. Moreover, genes involved in inhibition of WNT/β-catenin signaling, such as frizzled-related protein, dickkopf homolog 2, and β-catenin-interacting protein 1, showed down-regulation in diseased skin. The functional significance of these findings remains to be determined, since ex-

pression of CCND1, a well-known downstream target of WNT5 signaling, was down-regulated in lesioned skin.

Up to now, microarray reports of psoriatic skin or PBMCs from psoriasis patients have not mentioned IL-17, IL-22, or IL-23, although these interleukins appear to play an important role in psoriasis pathogenesis.[59,60] A feasible explanation for this might be that these cytokines were either not present on a particular chip[51] or were not detected because of low expression levels. In our own experiments on rheumatoid arthritis using oligonucleotide microarrays, IL-17, IL-22, and IL-23 expression was indeed mostly below or only slightly above the cut-off values for detection (M. Kunz, unpublished observation). However, a series of genes involved in IL-22/IL-23 mediated inflammation such as CXCL1/Gro-α, S100A7, S100A8, STAT3, IL-6, CCL22/MDC, SCYA20/MIP3α, and β-defensin 2 have been identified in the mentioned psoriasis microarray studies.[51,53,54,58] The functional significance of IL-23 was recently emphasized by a clinical trial, demonstrating successful treatment of psoriasis with a systemically administered anti-IL-12/23 antibody.[61]

Lupus Erythematosus

In recent years, a series of reports have been published on gene expression profiles of lupus erythematosus (LE).[62-64] In an earlier study, Maas and coworkers found no significant differences in gene expression patterns of PBMCs from patients with systemic LE (SLE), rheumatoid arthritis, multiple sclerosis (MS), and type I diabetes. But gene profiles of autoimmune patients showed significant differences compared with those of control patients, which had received influenza vaccination.[65] Enhanced expression was observed for 95 genes and reduced expression of 117 genes. Up-regulated genes involved receptor molecules, inflammatory molecules, and signal transduction molecules. Reduced expression was observed for genes with proapoptotic function (eg, TRADD and TRAF). These findings are supportive for the current concept of deregulated apoptosis in SLE.

The molecular relationship between SLE and MS, 2 independent autoimmune diseases, was analyzed in an independent study analyzing gene expression profiles of PBMC from both diseases.[66] When compared with healthy controls, a distinct pattern of 1031 genes for MS and 1146 genes for SLE, respectively, was identified. An overlapping pattern between SLE and MS, which separated both from control patients, included genes involved in apoptosis regulation (eg, TRAF5, caspase 8, BCL2, IER3 and IL1B), proinflammatory molecules, and genes involved in cellular proliferation and immune response (eg, IL11RA, VEGF, and CD19). The functional relevance of these findings was supported by the fact that transgenic mice overexpressing CD19 generate spontaneous antinuclear antibodies and DNA autoantibodies.[67] SLE alone was characterized by enhanced expression of genes involved in DNA damage and repair.

In a treatment study of 6 SLE patients suffering from lupus nephritis, half of the patients received intravenous methylprednisolone plus cyclophosphamide, and the other half was left untreated.[68] The majority of 151 differentially expressed genes showed up-regulation after treatment. These genes were involved in apoptosis, cell cycle regulation, and DNA repair/replication. Some of the genes have already been linked to LE, such as Fc fragment, immunoglobulin G, cytochrome c, p53, and CD22. This study provided evidence that common immunosuppressive treatments impact on mechanisms involved in LE pathogenesis.

In an elegant approach, Xu and coworkers showed that peripheral blood CD4+ T-cells from lupus patients are resistant to activation-induced cell death.[69] Microarray analysis of cell death resistant cells identified a specific gene cluster with high expression of cyclooxygenase-2 (COX-2). In functional assays, COX-2 inhibitors such as colecoxib and niflumic acid rendered resistant CD4+ T-cells susceptible to apoptosis, which was associated with activated Fas signaling and decreased expression of c-FLIP. On the basis of these findings, the authors concluded that COX-2 inhibitors may be used for the treatment of SLE. Indeed, there is a certain body of evidence that LE patients may profit from treatment with COX-2 inhibitors.[70]

A major breakthrough in the understanding of lupus pathogenesis came from a series of recent microarray studies, identifying a so-called interferon signature.[71-77] In a comprehensive study by Baechler and coworkers, gene expression profiles of peripheral blood cells of 48 SLE patients and 42 controls were analyzed.[71] A gene signature was identified in lupus patients, which showed significant overlap with that of IFN-α/β stimulated PBMC of normal controls. Twenty-three of 161 differentially expressed genes between lupus and control patients were IFN-regulated. Similar results were obtained in a microarray study on early onset lupus.[74] In the latter study, it could be shown that high-dose intravenous corticosteroid therapy, a common treatment of systemic lupus, significantly influenced the interferon gene signature. Genes that have been identified as interferon-response genes were IFIT1 (interferon-induced with tetratricopeptide repeats 1), IFI44 (interferon induced, hepatitis C-associated microtubular aggregate protein), MX1 (myxovirus resistance 1), OAS1, and OAS2. Further evidence for a particular role of type I IFNs in lupus pathogenesis was provided by high IFN serum levels of lupus patients, lupus-like symptoms in IFN-α treated patients, and a relatively benign course of the disease in a mouse model with defective IFN receptors.[78-80] Interestingly, plasmacytoid dendritic cells, which are enriched in skin lesions of LE patients, are major producers of IFN-α. More recent investigations found that plasma from lupus but not from rheumatoid arthritis patients induced IFN-α regulated genes in the WISH epithelial cell line and that plasma IFN-α levels correlated with anti-RNA binding protein (RBP)-specific autoantibodies.[81] Moreover, in proteome analyses using antibody microarrays, lupus patients showed enhanced expression of a set of 12 chemokines, many of which are regulated by type I IFNs.[82] Among these were CCL2 (MCP-1), CXCL10 (IP-10), CXCL9 (MIG), and CXCL11 (I-TAC).

Systemic Sclerosis

Systemic sclerosis (SSc) or systemic scleroderma presents as a progressive sclerosis of the skin often accompanied by

internal organ involvement. A series of different approaches have been used to identify genes involved in this disease. Many of the earlier studies used in vitro cultivated fibroblasts from SSc and control patients.[83] By use of differential display technology, enhanced expression of extracellular matrix molecules like fibronectin receptor, fibrosin, nexin-1 and insulin-like growth factor binding protein (IGFBP)-5 were identified. In a more recent microarray study, gene expression profiles of skin biopsies from SSc patients showed significant differences from those of healthy controls.[84] Cluster analysis identified so-called collagen I, B lymphocyte, cell adhesion and extracellular matrix, smooth muscle, and T-cell clusters, named after major genes within these clusters. To further substantiate these findings, gene patterns from SSc skin biopsies were compared with those of dermal microvascular endothelial cells, HS578T myofibroblast-like cells, B lymphocytes, dermal fibroblasts from involved and uninvolved SSc skin, and fibroblasts from normal controls. Indeed, many of the highly expressed genes in SSc skin biopsies were also significantly expressed in fibroblasts, endothelial cells, and B cells. Differentially expressed genes between SSc and normal control skin included calreticulin (CALR), collagen type XV α1 (COL15A1), nidogen 2 (NID2), connective tissue growth factor (CTGF). Differential expression characteristic for the B-lymphocyte signature included CD53, IGL, and IGHG3.

To detect early molecular events in SSc, gene expression profiles were generated from fibroblasts from skin biopsies taken from clinically uninvolved skin of 21 SSc patients and 18 healthy controls.[85] A 16,600 element oligonucleotide microarray was used. Differentially expressed genes were significantly enriched in the gene ontology categories of collagen (cat. no. 5581), extracellular matrix (cat. no. 5201), and complement activation (cat. no. 6956). Significant differences in gene expression were observed for COL7A1, COL18A1 (endostatin), COMP (cartilage oligomeric matrix protein), CD44, and 5 metallothionein genes. Authors suggested that COL7A1 and COL18A1 might be regarded as biomarkers for early onset of SSc. In line with this, a correlation between disease activity and serum COL18A1 levels has been described in SSc patients.[86]

A hallmark of SSc is pulmonary fibrosis. In a recent proteome approach, bronchoalveolar lavage fluid (BAL) from patients suffering from pulmonary sarcoidosis, pulmonary fibrosis associated with SSc, or idiopathic pulmonary fibrosis was analyzed.[87] BAL fluid proteins were identified by mass spectrometry after separation by 2D gel electrophoresis. Proteins which showed enhanced expression in SSc compared with idiopathic pulmonary fibrosis included α1-B glycoprotein, complement C3β, α1-antitrypsin, and haptoglobin β. Another set of proteins showed enhanced expression in SSc compared with sarcoidosis, namely prothrombin, thioredoxin, peroxisomal antioxidant enzyme, calgranulin, and thioredoxin peroxidase 2. Thrombin is a known mitogen for fibroblasts and enhanced thrombin levels had been demonstrated in SSc BAL fluid in earlier reports.[88] Thioredoxin expression had been shown in a rat model of oxidant-induced pulmonary fibrosis.[89] Taken together, the presented genome and proteome analyses are suggestive for an important role of B-cells, endothelial cells, and the coagulation system in the pathogenesis of SSc, besides that of fibroblasts.

Conclusion

Microarray technology is now widely used for gene expression studies of skin diseases. Many of the currently available microarray data have provided deeper insights into the pathogenesis of these diseases, which is particularly true for malignant melanoma, psoriasis, and LE. It should be kept in mind that data from different studies addressing the same disease may often not directly be compared because of the use of different tissues, microarray technologies, statistical methods, or cut-off values. Moreover, the biological variability of experiments in humans can only be overcome when large enough sample numbers are included, which was often not the case in earlier microarray studies. Larger sample numbers combined with sophisticated biostatistical analyses might in future help to identify subgroups of patients, which could pave the way for new more individualized treatment approaches.

Many of the findings from gene expression studies may now be validated using RNA interference technology, which allows the specific knockdown of individual genes or pathways.[90] Recent identification of so-called microRNAs, which are able to regulate several hundreds of genes at a time, may even allow to influence gene patterns or biological processes.[91] As emphasized recently, future investigations should also include different splice variants of genes, because these may have different and sometimes opposing functions.[92] Because many of the mentioned skin diseases have a strong genetic background, analyses addressing SNPs will be a further focus of future research.[15]

References

1. [No authors listed]: To affinity and beyond. Nat Genet 14:367-370, 1996.
2. Fodor SP, Read JL, Pirrung MC, et al: Light-directed, spatially addressable parallel chemical synthesis. Science 251:767-773, 1991
3. Lockhart DJ, Dong H, Byrne MC, et al: Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 14: 1675-1680, 1996
4. Duggan DJ, Bittner M, Chen Y, et al: Expression profiling using cDNA microarrays. Nat Genet 21:10-14, 1999 (suppl)
5. Reverter A, McWilliam SM, Barris W, et al: A rapid method for computationally inferring transcriptome coverage and microarray sensitivity. Bioinformatics 21:80-89, 2004
6. Draghici S, Khatri P, Eklund AC, et al: Reliability and reproducibility issues in DNA microarray measurements. Trends Genet 22:101-109, 2006
7. Tan PK, Downey TJ, Spitznagel EL Jr, et al: Evaluation of gene expression measurements from commercial microarray platforms. Nucleic Acids Res 31:5676-5684, 2003
8. MAQC Consortium, Shi L, Reid LH, et al: The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 24:1151-1161, 2006

9. Chee M, Yang R, Hubbell E, et al: Accessing genetic information with high-density DNA arrays. Science 274:610-614, 1996
10. Hacia JG, Brody LC, Chee MS, et al: Detection of heterozygous mutations in BRCA1 using high density oligonucleotide arrays and two-colour fluorescence analysis. Nat Genet 14:441-447, 1996
11. Wen WH, Bernstein L, Lescallett J, et al: Comparison of TP53 mutations identified by oligonucleotide microarray and conventional DNA sequence analysis. Cancer Res 60:2716-2722, 2000
12. Lipshutz RJ, Fodor SPA, Gingeras TR, et al: High density synthetic oligonucleotide arrays. Nat Genet 21:20-24, 1999 (suppl)
13. Shastry BS: SNP alleles in human disease and evolution. J Hum Genet 47:561-566, 2002
14. Matsuzaki H, Loi H, Dong S, et al: Parallel genotyping of over 10,000 SNPs using a one-primer assay on a high-density oligonucleotide array. Genome Res 14:414-425, 2004
15. Meaburn E, Butcher LM, Liu L, et al: Genotyping DNA pools on microarrays: Tackling the QTL problem of large samples and large numbers of SNPs. BMC Genomics 6:52, 2005
16. Müller A, Holzmann K, Kestler HA: Visualization of genomic aberrations using Affymetrix SNP arrays. Bioinformatics 23:496-497, 2007
17. Kim S, Misra A: SNP genotyping: Technologies and biomedical applications. Annu Rev Biomed Eng 9:289-320, 2007
18. Chung CH, Levy S, Chaurand P, et al: Genomics and proteomics: Emerging technologies in clinical cancer research. Crit Rev Oncol Hematol 61:1-25, 2006
19. Haab BB, Dunham MJ, Brown PO: Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Biol 2:research0004, 2001
20. Quackenbush J: Microarray data normalization and transformation. Nat Genet 32 Suppl:496-501, 2002
21. Draghici S: Statistical intelligence: effective analysis of high-density microarray data. Drug Discov Today 7:S55-S63, 2002 (suppl 11)
22. Irizarry RA, Bolstad BM, Collin F, et al: Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 31:4:e15, 2003
23. Bolstad BM, Irizarry RA, Astrand M, et al: A comparison of normalization for high density oligonucleotide array data based on variance and bias. Bioinformatics 19:185-193, 2003
24. Kaufman L, Rousseeuw PJ: Finding Groups in Data. An Introduction to Cluster Analysis. New York, John Wiley & Sons, 1990
25. Bishop CM: Neural Networks for Pattern Recognition. Oxford, Oxford University Press, 1995
26. Brown MP, Grundy WN, Lin D, et al: Knowledge-based analysis of microarray gene expression data by using support vector machines. Proc Natl Acad Sci U S A 97:262-267, 2000
27. Eisen MB, Spellman PT, Brown PO, et al: Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 95:14863-14868, 1998
28. Kohonen T: Comparison of SOM point densities based on different criteria. Neural Comput 11:2081-2095, 1999
29. Ramaswamy S, Golub TR: DNA microarrays in clinical oncology. J Clin Oncol 20:1932-1941, 2002
30. Ramaswamy S, Ross KN, Lander ES, et al: A molecular signature of metastasis in primary solid tumors. Nat Genet 33:49-54, 2003
31. Van 't Veer LJ, Dai H, van de Vijver MJ, et al: Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530-536, 2002
32. Wang Y, Klijn JG, Zhang Y, et al: Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 365:671-679, 2005
33. DeRisi J, Penland L, Brown PO, et al: Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 14:457-460, 1996
34. Clark EA, Golub TR, Lander ES, et al: Genomic analysis of metastasis reveals an essential role for RhoC. Nature 406:532-535, 2000
35. Kunz M, Koczan D, Ibrahim S, et al: Differential expression of thrombospondin 2 in primary and metastatic malignant melanoma. Acta Derm Venereol 82:163-169, 2002
36. Bittner M, Meltzer P, Chen Y, et al: Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 406: 536-540, 2000
37. Weeraratna AT, Jiang Y, Hostetter G, et al: Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell 1:279-288, 2002
38. Carr KM, Bittner M, Trent JM: Gene-expression profiling in human cutaneous melanoma. Oncogene 22:3076-3080, 2003
39. Becker B, Roesch A, Hafner C, et al: Discrimination of melanocytic tumors by cDNA array hybridization of tissues prepared by laser pressure catapulting. J Invest Dermatol 122:361-368, 2004
40. Hagg C, Nosrati M, Sudilovsky D, et al: The gene expression signatures of melanoma progression. Proc Natl Acad Sci U S A 102:6092-6097, 2005
41. Winnepenninckx V, Lazar V, Michiels S, et al: Gene expression profiling of primary cutaneous melanoma and clinical outcome. J Natl Cancer Inst 98:472-482, 2006
42. Kunz M, Moeller S, Koczan D, et al: Mechanisms of hypoxic gene regulation of angiogenesis factor Cyr61 in melanoma cells. J Biol Chem 278:45651-45660, 2003
43. Straume O, Akslen LA: Importance of vascular phenotype by basic fibroblast growth factor, and influence of the angiogenic factors basic fibroblast growth factor/fibroblast growth factor receptor-1 and ephrin-A1/EphA2 on melanoma progression. Am J Pathol 160:1009-1019, 2002
44. Jaeger J, Koczan D, Thiesen HJ, et al: Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. Clin Cancer Res 13:806-15, 2007
45. O'Driscoll L, McMorrow J, Doolan P, et al: Investigation of the molecular profile of basal cell carcinoma using whole genome microarrays. Mol Cancer 5:74, 2006
46. Rubin AI, Chen EH, Ratner D: Basal-cell carcinoma. N Engl J Med 353:2262-2269, 2005
47. Nindl I, Dang C, Forschner T, et al: Identification of differentially expressed genes in cutaneous squamous cell carcinoma by microarray expression profiling. Mol Cancer 5:30, 2006
48. Schön MP, Boehncke WH: Psoriasis. N Engl J Med 352:1899-1912, 2005
49. Bos JD, de Rie MA: The pathogenesis of psoriasis: Immunological facts and speculations. Immunol Today 20:40-46, 1999
50. Krueger JG, Bowcock A: Psoriasis pathophysiology: C concepts of pathogenesis. Ann Rheum Dis 64:ii30-ii36, 2005 (suppl 2)
51. Bowcock AM, Shannon W, Du F, et al: Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. Hum Mol Genet 10:1793-1805, 2001
52. Oestreicher JL, Walters IB, Kikuchi T, et al: Molecular classification of psoriasis disease-associated genes through pharmacogenomic expression profiling. Pharmacogenomics J 1:272-287, 2001
53. Quekenborn-Trinquet V, Fogel P, Aldana-Jammayrac O, et al: Gene expression profiles in psoriasis: analysis of impact of body site location and clinical severity. Br J Dermatol 152:489-504, 2005
54. Zhou X, Krueger JG, Kao MC, et al: Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. Physiol Genomics 13:69-78, 2003
55. Koczan D, Guthke R, Thiesen HJ, et al: Gene expression profiling of peripheral blood mononuclear leukocytes from psoriasis patients identifies new immune regulatory molecules. Eur J Dermatol 15:251-257, 2005
56. Li G, Domenico J, Lucas JJ, et al: Identification of multiple cell cycle regulatory functions of p57Kip2 in human T lymphocytes. J Immunol 173:2383-2391, 2004
57. Rappersberger K, Komar M, Ebelin ME, et al: Pimecrolimus identifies a common genomic anti-inflammatory profile, is clinically highly effective in psoriasis and is well tolerated. J Invest Dermatol 119:876-887, 2002
58. Reischl J, Schwenke S, Beekman JM, et al: Increased expression of Wnt5a in psoriatic plaques. J Invest Dermatol 127:163-169, 2007
59. Nickoloff BJ: Cracking the cytokine code in psoriasis. Nat Med 13:242-244, 2007
60. Zheng Y, Danilenko DM, Valdez P, et al: Interleukin-22, a T(H)17

cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 445:648-51, 2007
61. Krueger GG, Langley RG, Leonardi C, et al: A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. N Engl J Med 356:580-592, 2007
62. Qing X, Putterman C: Gene expression profiling in the study of the pathogenesis of systemic lupus erythematosus. Autoimmune Rev 3:505-509, 2004
63. Centola M, Frank MB, Bolstad AI, et al: Genome-scale assessment of molecular pathology in systemic autoimmune diseases using microarray technology: A potential breakthrough diagnostic and individualized therapy-design tool. Scand J Immunol 64:236-242, 2006
64. Baechler EC, Batliwalla FM, Reed AM, et al: Gene expression profiling in human autoimmunity. Immunol Rev 210:120-137, 2006
65. Maas K, Chan S, Parker J, et al: Cutting edge: molecular portrait of human autoimmune disease. J Immunol 169:5-9, 2002
66. Mandel M, Gurevich M, Pauzner R, et al: Autoimmunity gene expression portrait: specific signature that intersects or differentiates between multiple sclerosis and systemic lupus erythematosus. Clin Exp Immunol 138:164-170, 2004
67. Sato S, Hasegawa M, Fujimoto M, et al: Quantitative genetic variation in CD19 expression correlates with autoimmunity. J Immunol 165:6635-6643, 2000
68. Pereira E, Tamia-Ferreira MC, Cardoso RS, et al: Immunosuppressive therapy modulates T lymphocyte gene expression in patients with systemic lupus erythematosus. Immunology 113:99-105, 2004
69. Xu L, Zhang L, Yi Y, et al: Human lupus T cells resist inactivation and escape death by upregulating COX-2. Nat Med 10:411-415, 2004
70. Østensen M, Villiger PM: Nonsteroidal anti-inflammatory drugs in systemic lupus erythematosus. Lupus 10:135-139, 2001
71. Baechler EC, Batliwalla FM, Karypis G, et al: Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 100:2610-2615, 2003
72. Han GM, Chen SL, Shen N, et al: Analysis of gene expression profiles in human systemic lupus erythematosus using oligonucleotide microarray. Genes Immun 4:177-186, 2003
73. Crow MK, Kirou KA, Wohlgemuth J: Microarray analysis of interferon-regulated genes in SLE. Autoimmunity 36:481-490, 2003
74. Bennett L, Palucka AK, Arce E, et al: Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 197:711-723, 2003
75. Qing X, Putterman C: Gene expression profiling in the study of the pathogenesis of systemic lupus erythematosus. Autoimmun Rev 3:505-509, 2004
76. Crow MK: Interferon pathway activation in systemic lupus erythematosus. Curr Rheumatol Rep 7:463-468, 2005
77. Pascual V, Farkas L, Banchereau J: Systemic lupus erythematosus: All roads lead to type I interferons. Curr Opin Immunol 18:676-682, 2006
78. Ytterberg SR, Schnitzer TJ: Serum interferon levels in patients with systemic lupus erythematosus. Arthritis Rheum 25:401-406, 1982
79. Ronnblom LE, Alm GV, Oberg K: Autoimmune phenomena in patients with malignant carcinoid tumors during interferon-alpha treatment. Acta Oncol 30:537-540, 1991
80. Santiago-Raber ML, Baccala R, Haraldsson KM, et al: Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J Exp Med 197:777-788, 2003
81. Hua J, Kirou K, Lee C, et al: Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. Arthritis Rheum 54:1906-1916, 2006
82. Bauer JW, Baechler EC, Petri M, et al: Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. PLoS Med 3:e491, 2006
83. Strehlow DR: The promise of transcription profiling for understanding the pathogenesis of scleroderma. Curr Rheumatol Rep 2:506-511, 2000
84. Whitfield ML, Finlay DR, Murray JI, et al: Systemic and cell type-specific gene expression patterns in scleroderma skin. Proc Natl Acad Sci U S A 100:12319-12324, 2003
85. Tan FK, Hildebrand BA, Lester MS, et al: Classification analysis of the transcriptosome of nonlesional cultured dermal fibroblasts from systemic sclerosis patients with early disease. Arthritis Rheum 52:865-876, 2005
86. Hebbar M, Peyrat JP, Hornez L, et al: Increased concentrations of the circulating angiogenesis inhibitor endostatin in patients with systemic sclerosis. Arthritis Rheum 43:889-893, 2000
87. Rottoli P, Magi B, Perari MG, et al: Cytokine profile and proteome analysis in bronchoalveolar lavage of patients with sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis. Proteomics 5:1423-1430, 2005
88. Ohba T, McDonald JK, Silver RM, et al: Scleroderma bronchoalveolar lavage fluid contains thrombin, a mediator of human lung fibroblast proliferation via induction of platelet-derived growth factor alpha-receptor. Am J Respir Cell Mol Biol 10:405-412, 1994
89. Ishii Y, Hirano K, Morishima Y, et al: Early molecular and cellular events of oxidant-induced pulmonary fibrosis in rats. Toxicol Appl Pharmacol 167:173-181, 2000
90. Milhavet O, Gary DS, Mattson MP: RNA interference in biology and medicine. Pharmacol Rev 55:629-648, 2003
91. Inoue K: Mechanisms of microRNA-mediated gene regulation in animal cells. Trends Genet 23:243-249, 2007
92. Fathman CG, Soares L, Chan SM, et al: An array of possibilities for the study of autoimmunity. Nature 435:605-611, 2005