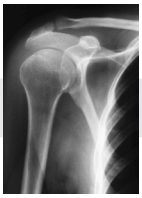

Updates on High-Throughput Molecular Profiling for the Study of Rheumatoid Arthritis

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Abstract

The completion of the human genome mapping project has provided the necessary backdrop for the development of high-throughput array technologies. In contrast to the reductionist approach to studying the role of a particular molecule in a specific pathway, these technologies enable us to conduct a comprehensive survey of various molecular profiles across different cell types and individuals, placing us one step closer to understanding integrative regulatory networks and the workings of the cell as a whole.

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Systemic autoimmune rheumatic diseases are complex and heterogeneous, with differences in age of onset, disease manifestation, prognosis, and response to treatment. One of the most common rheumatic diseases is rheumatoid arthritis, a systemic autoimmune disease affecting 1% of the world population. Patients are afflicted with erosive arthritis and suffer from chronic joint pain. The disease also has systemic manifestations, including increased risks of developing lymphoma, infection, and atherosclerotic heart disease.

Recent advances in biomedical sciences have provided us with an unforeseen number of new arsenals against RA. However, we have yet to integrate patients' unique biological milieu into treatment strategy while using medications that suppress the immune system non-specifically and carry small, but serious, side effects. It is with immense hope that these recent advances in high-throughput molecular profiling technologies will transform the quest for individualized medicine from a scientific fantasy to a realistic challenge. Various high-throughput molecular profiling technologies have been developed and applied to autoimmune diseases [1]. This review will focus on the main technologies that contribute to the understanding of RA. It will not include the study of RA using animal models; for a more comprehensive discussion of the technology, see the excellent review by Fathman et al. [2].

DNA-related technologies

Complementary DNA technology involves reverse-transcribing mRNAs into cDNAs, which are then labeled with fluorescent dyes and hybridized onto arrays consisting of DNA fragments or oligonucleotides on a solid support. The fluorescent intensity of a spot is directly related to the degree of transcriptional activation of the corresponding gene. This pattern of varying brightness is analyzed by sophisticated comparison and clustering programs to generate the transcription profile of differential gene expression between various samples.

Early application of this technology provided clear evidence of RA as a heterogeneous disease. Work by Kraan et al. [3] using synovial samples from 15 RA patients showed that the gene expression profile fell into two molecularly distinct subsets. One subset showed a high level of inflammatory gene expression, which included activation of the STAT-1 pathway, whereas the other was reminiscent of osteoarthritis with high expression levels of genes involved in fibroblast differentiation and extracellular matrix remodeling, but low on inflammatory gene transcription. More recently, Leguerre and co-workers [4] showed that this technology can be used to differentiate between treatment responders from non-responders. They tested 20 differentially regulated transcripts in a group of 20 patients, which predicted treatment response with 90% sensitivity and 70% specificity. Although further validation is needed, this study suggests that patient-specific treatment stratification may not be far into the future.

Rheumatoid arthritis is a heterogeneous disease with different molecular subtypes

The cDNA array, in addition to its potential application as a biomarker for disease and treatment response, has also provided significant insights into the pathogenesis of RA [5-8]. Genes found to be differentially expressed in RA include those involved in apoptosis, inflammation, cell trafficking, and angiogenesis [9]. While the importance of various biological pathways has been

RA = rheumatoid arthritis
cDNA = complementary DNA

Table 1. Summary of high-throughput microarray technologies available to address biological questions pertinent for the study of autoimmune diseases

Question	Technology	Description	Limitations
What is the difference in gene expression between two sets of conditions?	cDNA microarray	DNA fragments or oligonucleotides are immobilized on slides and probed with cDNAs reverse transcribed from mRNAs in patients' samples	Unable to detect splice variants
What is the genetic variation between different populations?	Single nucletotide polymorphism analysis	Various platforms are available, including allele-specific primer hybridization, extension or ligation, and sequencing	Unclear significance of non-functional polymorphisms
What is the difference in protein expression?	Antibody microarray	Antibodies to specific proteins are immobilized on slides and probed with patients' samples to quantitate the target proteins of interest	Limited commercially available antibodies and problem with antibody cross-reactivity
What is the difference in antibody profile?	Autoantigen microarray	Purified proteins or peptides are deposited on slides and probed with patients' samples to identify the presence of targeting antibody.	Requires prior knowledge of autoantigens
What is the difference in protein phosphorylation under different conditions?	Multiparameter phosphoprotein flow cytometry	Multiple protein phosphorylation states in a single cell can be detected using phospho-specific antibodies and multiparameter flow cytometry.	Not all phospho-epitopes are detectable due to antibody availability. Potential antibody cross-reactivity.

speculated, the precise role for many of these molecules in this complex biomolecular network is still unclear and awaits further elucidation.

Another important tool in DNA-based analysis is the study of genetic variations between individuals by single nucleotide polymorphisms. A genetic contribution to the susceptibility of developing RA was first suspected based on clustering of the disease in families, and later confirmed with concordance studies of monozygotic twins (15%–30%) [10]. This genetic susceptibility was found to be largely attributable to class II human leukocyte antigen, particularly to HLA-DR4 and related alleles that share a region of the DR beta-chain that forms one side of the peptide binding groove [11]. No other risk alleles were identified for more than two decades, when PTPN22 was found using single nucleotide polymorphism [12]. More recently, additional risk alleles have been identified using this more comprehensive and detailed genetic analysis, adding Stat4, TRAF1, and the complement component 5 to the family of genes associated with the development of RA [13,14].

Early study shows that differences in transcript profiles can predict treatment response to TNF-antagonists

Protein-based technologies

There is a myriad of approaches to identify proteins that are differentially expressed or modified in RA patients but not in healthy controls. Earlier studies using two-dimensional gel electrophoresis or liquid chromatography in tandem with mass spectrometry led to the identification of differentially expressed proteins such as the S100 family of calcium-binding protein that was particularly abundant in RA synovial fluid [15,16]. However, because of the laborious protocol and poor sensitivity, there was great interest in developing high-throughput protein analysis.

There are now dazzling numbers of protein analysis platforms, including multiplex western blots and various permutations of protein microarrays. Multiplex western blot was used by Lorenz et al. [17] to examine proteins differentially expressed between RA and osteoarthritis synovial tissues. Their study showed that changes in gene expression by proteome and trascriptome were frequently discordant. In addition, significant differences in protein level were found for Stat1, p47phox, cathepsin D, and

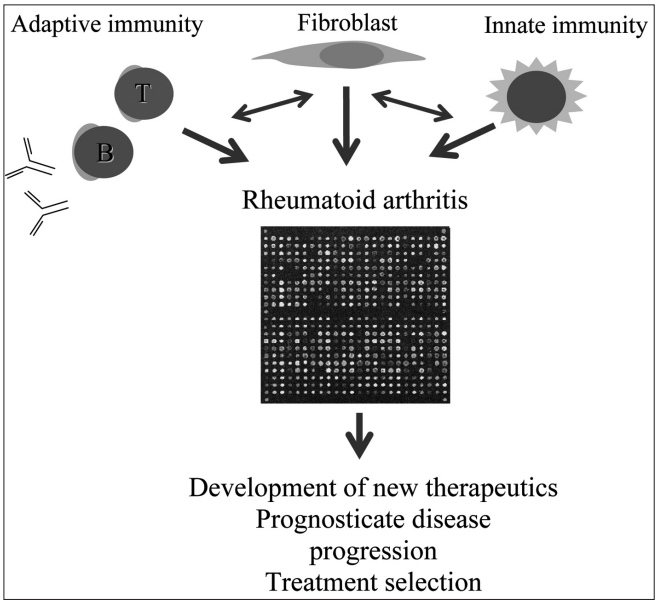

Figure 1. The potential path from pathology to cure. Chronic synovitis in rheumatoid arthritis is the result of a complex interplay between various components of the immune system and synovial fibroblasts. These intricate interactions involve both cytokine network and direct cell contacts, and occur both systemically and locally in the joint. Genome-wide study is being performed using various genomic and proteomic technologies with the goal of developing new treatment strategy, prognosticate disease progression, and tailor treatment options to the specific biology of individual patients.

HLA = human leukocyte antigen

MnSOD, adding them to the growing list of molecules that may be important for RA pathogenesis.

As the array technology matures and improves, there are now various array-based platforms that allow rapid analysis of protein composition. These assays commonly involve printing protein probes in geographically distinct spots onto a solid support, and have been used to probe antigen, antibody, and cytokine profiles in RA [18]. Using a synovial antigen array containing 225 peptide and proteins representing candidate RA autoantigens, Hueber and team [19] profiled autoreactive B cell response and demonstrated heterogeneity in the specificity of autoantibodies among RA patients. Extension of this study using multiplex cytokine array showed high serum levels of cytokines including tumor necrosis factor-alpha, interleukin-1 beta, interleukins-2, 4, 6, 13 and 15, and granulocyte macrophage colony-stimulating factor in association with increased autoantibody reactivity against citrullinated epitopes [20]. Interestingly, different processes may be involved in the beginning and later stages of RA, as the levels of cytokines seen at disease onset are decreased in established disease [21].

Distinct autoantibody and cytokine signatures are found in rheumatoid arthritis patients

Conclusion and future direction

The application of genomic and proteomic approaches to the study of RA has led to a greater understanding of the disease. Remaining challenges include the issue of important splice variants in distinct cell types that are not accounted for using the current cDNA microarray. There is also a dearth of high quality monoclonal antibodies for comprehensive analysis of the proteome, as well as insufficient bioinformatics tools to analyze the increasingly complex datasets. Several commercial bodies are developing technologies to address the problem of distinct splice variants via exon or single-nucleotide arrays. New ways to probe cellular function are also being developed, including phospho-specific flow cytometry for determining the phosphorylation state of proteins on a single cell level. It is hopeful that these promising technologies will lead to the discovery of new therapies as well as treatment individualization, and ultimately, to better patient care.

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