A genomic view of subtypes in rheumatoid arthritis: towards personalized medicine

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There is growing evidence that rheumatoid arthritis (RA) is a heterogeneous disease. The disease is generally referred to in terms of a group average, which may hamper the progress in understanding its genetic basis, pathogenic mechanisms and the treatment success for subsets of patients. Unfortunately, criteria for subtyping of patients, for example to select those patients who will benefit from a specific treatment, is currently lacking. Since, by definition, nearly every aspect of a disease phenotype should be represented in the pattern of genes and proteins that are expressed in the affected tissues and organs, molecular typing of patients is likely to yield useful classifiers for RA subtyping. This paper will describe novel developments in genomics and proteomics research for the identification of biomarkers for disease subclassification in RA. This information will also improve our understanding of the underlying biology of RA subtypes. Ultimately, this information will help clinicians to select subgroups of RA patients for optimal treatment.

The clinical presentation of patients with rheumatoid arthritis (RA) may reveal striking heterogeneity, with a spectrum ranging from mild cases to severe and erosive disease. Heterogeneity is already reflected in the classifying diagnosis for RA, which is based on the presence of four out of seven criteria, and thus indicates that different sets of criteria are applied to classify 'the same' disease [1,2].

The heterogeneous nature of RA is also reflected at the level of the distribution of lymphocytes in the rheumatoid synovium, which reveals a remarkably patient-specific organization level [3–5]. In approximately a quarter of RA patients, cellular infiltrates in the synovial tissue show a high degree of organization, resembling germinal center (GC)-like structures normally observed in secondary lymphoid organs. In the remainder of the patients, these GC-like structures are absent in the affected synovial tissues. These patients show either a diffuse lymphocytic infiltrate or an aggregated T- and B-cell infiltrate [6].

The variation in responsiveness to virtually any treatment modality in RA is consistent with the heterogeneous nature of the disease. For example, despite the highly beneficial effects of tumor necrosis factor (TNF) blocking in suppressing disease, clear efficacy appears to be limited to a subset of patients [7,8]. Owing to the observations that only approximately a quarter of the patients show a significant clinical improvement, according to the American College of Rheumatology (ACR)-70 criteria, and a significant proportion of patients do not respond at all to TNF blockade, additional effectors and/or pathways are thought to contribute to the disease. The relative contribution of the different effectors and/or disease pathways may vary between patients and, perhaps, between different stages of disease. Similar observations have been made for other therapies such as the treatment with cytotoxic T lymphocyte antigen (CTLA)4Ig, which blocks the interaction of CD80/86 on antigen-presenting cells with CD28 on T cells, and B-cell ablation therapy [9,10]. Hence, the cumulative data provide evidence that distinct pathogenic mechanisms contribute to disease in RA.

The heterogeneity probably has its origin in the multifactorial nature of the disease, whereby specific combinations of environmental factor(s) and a varying polygenic background are likely to influence not only susceptibility, but also the severity and disease outcome.

Given the destructive nature of the disease, it would be highly desirable to predict, at an early stage, the most beneficial treatment for the subgroup of patients at risk. If we rely solely on clinical or radiological manifestations, we will probably be responding too late in order to maximize protection. Unfortunately, important criteria to make selections of patients for optimal treatment and research purposes are currently lacking. Such criteria would be highly beneficial for patient stratification to assign homogeneous groups of patients for genetic studies and to improve the likelihood of observing efficacy of treatment. Ultimately, this may lead to a personalized form of medicine, whereby a specific therapy will be applied that is best suited to each individual patient.

Keywords: biomarkers, disease subtypes, genomics, heterogeneity, microarray, molecular profiling, personalized medicine, proteomics, rheumatoid arthritis

future medicine

Molecular profiling of RA

By definition, nearly every aspect of a disease phenotype should be represented in the pattern of genes and proteins that are expressed in the patient. The application of large-scale gene expression profiling using DNA microarrays (genomics) and sophisticated proteomics technology in blood and tissue samples from patients with RA allows an open-ended survey to identify comprehensively the fraction of genes and proteins that are specific to a disease subtype or phase. This molecular signature represents typically the contributions and interactions of specific factors and distinct cells that are associated with disease characteristics and subtypes, and thus defines the samples' unique biology. This review will mainly focus on novel developments in genomics and proteomics research for the identification of biomarkers for disease subclassification in RA.

Autoantibodies

For RA, the presence of specific autoantibodies against antigens containing one or more citrulline residues, the so called anticyclic citrulline peptide (CCP) antibodies and rheumatoid factor (RF), are instrumental in classifying the diagnosis [2,11].

Nielen and colleagues [12] and Rantapaa-Dahl-qvist and colleagues [13] analyzed the levels of RF and anti-CCP autoantibodies in dated serum samples from RA patients who were former blood donors. They reported that RF and anti-CCP autoantibodies were already present before the appearance of the first clinical signs of arthritis. From these studies, it is clear that production of these markers is an early process and that their appearance is predictive for the development of RA.

Large cohort studies demonstrated that anti-CCP antibodies, similar to RF, are present in approximately 80% of patients with established RA. By contrast, the healthy control group and the non-RA disease controls were only positive in a maximum of 1 and 5%, respectively, whereas the corresponding percentages of RF were markedly higher (10 and 20%, respectively) [14–19]. Together, these studies show that anti-CCP antibodies equal RF for sensitivity, but have far better specificity. The finding that approximately 40% of RF-negative patients are anti-CCP positive is consistent with the heterogeneous nature of the disease and supports additional diagnostic potential for anti-CCP antibodies [20].

Recent studies have demonstrated the use of anti-CCP as a valuable specific serological marker to distinguish RA from other non-erosive types of arthritis [12,13,21–23]. In addition, several studies have demonstrated the prognostic value of anti-CCP antibodies by their ability to predict the erosiveness of developing RA [24–28]. The predictive ability of anti-CCP has also been studied in combination with other disease parameters. Anti-CCP together with RF appeared the best prognosticator for the development of persisting RA [13,21,29]. It was also reported that a combination of anti-CCP antibodies with human leukocyte antigen (HLA) class DRB1 antigens is strongly associated with the future onset of RA, thereby predicting the future development of RA [19,22,30]. Thus, anti-CCP appears to identify a subgroup of patients with an increased chance of developing persistent and erosive disease.

These examples show the clinical value of autoantibody measurements in RA. Besides RF and anti-CCP, there are a number of other antibody specificities found to be present in RA [31]. The clinical value of most of these antibodies as single entities or in combination remains to be determined. The advent of high-throughput technologies that allow the parallel profiling of hundreds of proteins has dramatically transformed the multiplex detection and search for autoantibody specificities that might be important in diagnosis and disease management [32,33]. Such protein arrays enable profiling of the specificity of autoantibody responses against panels of peptides and proteins representing known autoantigens, as well as candidate autoantigens. Recently, the application of antigen microarrays revealed that serum from RA patients with markers of more severe disease (high C-reactive protein [CRP] levels and RF-seropositive patients) contain antibodies that bind preferentially to citrullinated epitopes, whereas RF-seronegative RA patients and patients with low CRP levels show antibody reactivity against native epitopes on the same and other proteins [33]. This technology holds the promise to define autoantibody signatures that define subsets of patients with different clinical disease subtypes and treatment responses. Hence, a combination of biomarkers that may include RF and anti-CCP could reflect different aspects of the disease process and, therefore, might be useful for evaluating prognosis in individual patients with early RA [30].

Protein markers

The emerging developments in proteomics technologies, based on mass spectrometry, automation, liquid handling systems, fractionation techniques and bioinformatics, together with the increased throughput and easier comparative expression analysis between samples, hold the promise to contribute to better diagnostics, prognostics and disease management. Protein profiling studies using surface-enhanced laser desorption/ionization (SELDI) mass spectrometry analysis of protein profiles revealed the abundant presence of S100A8 (calgranulin A/myeloid-related protein[MRP]8) and S100A9 (calgranulin B/MRP14) in sera and synovial fluids of RA patients [34–36]. S100A8 and S100A9 belong to a new class of inflammatory mediators that are released by activated monocytes upon interaction with activated endothelial cells under inflammatory conditions [37]. One of the functions of the heterodimer complex is to mediate leukocyte migration and adhesion to the vascular endothelium. These markers are not specific for RA since they have also been found in other inflammatory conditions, such as cystic fibrosis, chronic bronchitis, Crohn's disease and ulcerative colitis [38–40]. However, by application of 2-dimensional liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS), Liao and colleagues were able to demonstrate that S100A8 and S100A9, in combination with CRP and S100A12, were significantly increased in the serum of patients with erosive compared with nonerosive RA [41].

In addition, there are several approaches for the parallel identification and quantification of individual proteins using bead and microarray platforms. Hitchon and colleagues used a multitude of color-coded microspheres, conjugated with a monoclonal antibody specific for the target protein to multiplex cytokine measurements in the plasma of RA patients [42]. Based on the multiplex measurement of cytokines, they demonstrated that RA patients are clustered in subgroups. A 'mild' RA subgroup had higher CCL4 (macrophage inflammatory protein [MIP]β), CXCL8 (interleukin [IL]-8), IL-1, IL-12, IL-17, IL-5 and IL-10 levels, lower IL-6, interferon (IFN)-γ, granulocyte macrophage colony stimulating factor (GM-CSF) and IL-4 levels, less anti-CCP positivity and lower anti-CCP titers, but similar erythrocyte sedimentation rate, CRP and joint counts, compared with the severe RA group [42]. Thus, the integration of autoantibody status with cytokine profile may assist with prognostication and eventual treatment decisions in RA.

Haab and colleagues have explored the use of antibody arrays, which have had significant applications in cancer research [43]. The antibody arrays, which have not yet been applied in rheumatological research, can be used for protein profiling, biomarker identification, protein characterization and the detection of protein-post-translational modifications. The application of antibody arrays in diseases such as RA would be highly effective for the discovery of novel protein biomarkers.

Large-scale gene expression profiling in RA

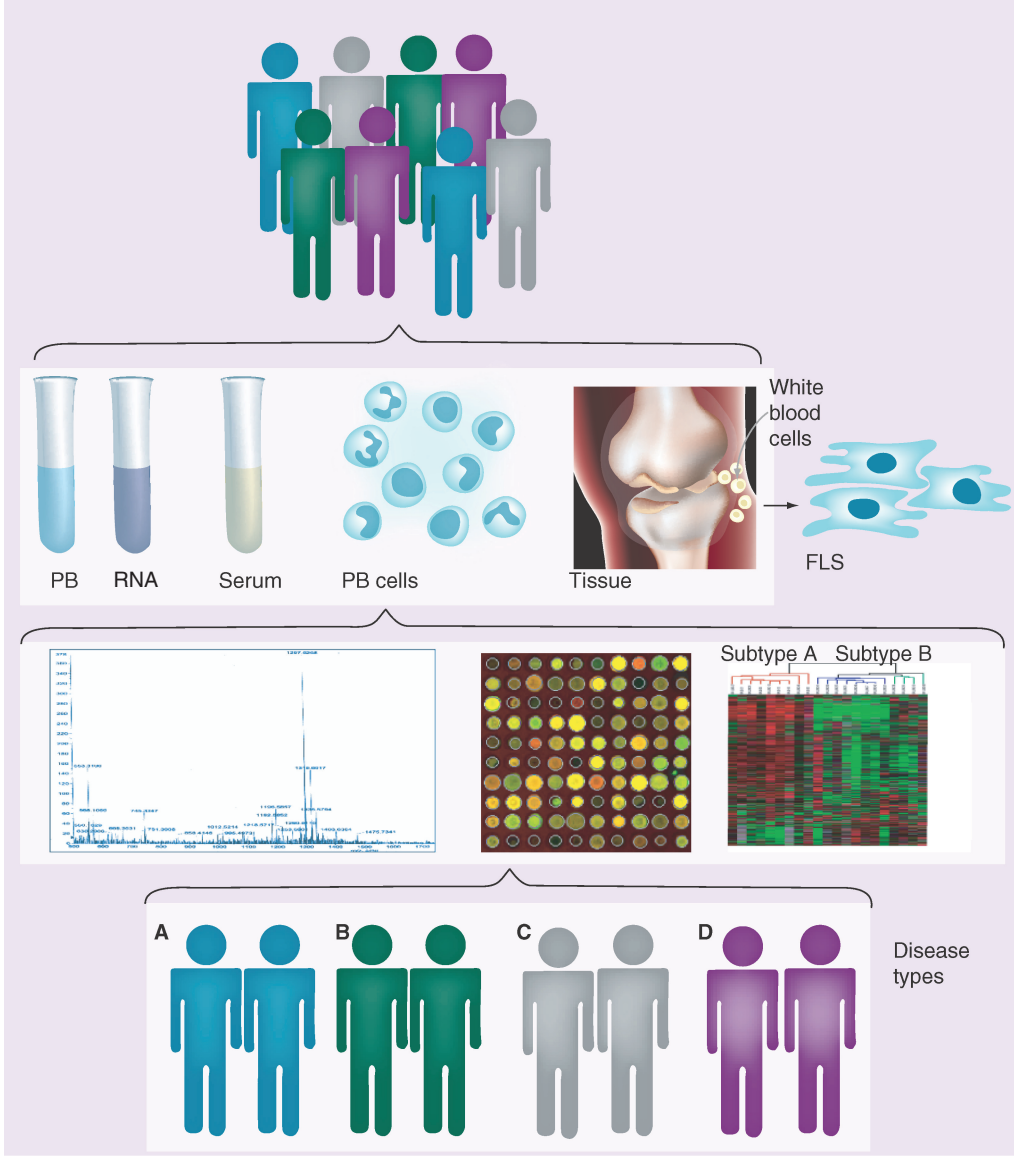
As mentioned previously, a powerful way to gain insight into the molecular complexity of cells and tissues has arisen from DNA microarray technology, which allows an open-ended survey to comprehensively identify the fraction of genes that are differentially expressed among patients with clinically defined RA. The differentially expressed gene sets may then be used as disease classifiers and surrogate markers for the measurement of a clinical end point and the involvement of particular biological pathways in the disease (**Figure 1**). Such an approach has proven useful in cancer research for the identification of classifiers for disease outcome, metastasis and underlying pathways [44,45].

Heterogeneity in rheumatoid tissue revealed by gene expression profiling

One of the first studies on gene expression profiles in RA biopsies was performed using a combination of subtractive hybridization and high-density complementary (c)DNA arrays [46]. This report showed an increased expression of genes involved in chronic inflammation, such as Igs and HLA-DR in RA synovium when compared with normal synovium. However, since the authors used pooled tissues from three RA patients and three healthy controls, heterogeneity in RA could not be considered.

Devauchelle and coworkers studied the differences in gene expression profiles between RA (number of patients [n] = 5) and osteoarthritis (OA; n = 10) synovial tissue [47]. Although the authors could make a selection of 63 differentially expressed genes in order to correctly classify all RA and OA samples, they did not address the issue of differences between patients.

Figure 1. A schematic outline for disease subtyping in rheumatoid arthritis.



Rheumatoid arthritis patients reveal a striking heterogeneity based on clinical, biological and molecular criteria. The categorization of patients is expected to be of utmost importance for decision making in clinical practice. Recent developments in high-throughput screening technologies have provided the opportunity to characterize patients based on their molecular profile. The application of technologies such as transcriptomics, proteomics and metabolomics allows us to determine the molecular description of an individual patient. When associated with clinical readouts, we could select the clinically useful molecular markers and apply these in day-to-day clinical practice.

The procedure starts with collecting different types of material, such as serum, PB cells, RNA from blood (using, e.g., PAXgene tubes), biopsies and FLS from the same patients. This material can than be analyzed by mass spectrometry, DNA-, protein- or antibody-arrays. Subsequently, computational algorithms will be applied to select subgroups of patients. For example, based purely on differences in a molecular profile; based on the presence or absence of a known response signature for the target of interest, or based on markers that are significantly associated with a clinical phenotype or disease outcome. These data help to elucidate the distinct pathological mechanisms at play that can explain the interpatient variation in clinical presentation, disease progression and treatment response. Knowledge of the differential pathogenic mechanisms will help the identification of new drug targets for selected patient subgroups. As an example, the authors hypothesize that a homogeneous subgroup of patients could be stratified based on a specific molecular portrait that correlates with the responder/nonresponder status of a targeted therapy.

FLS: Fibroblast-like synoviocytes; PB: Peripheral blood.

Recently, Lindberg and coworkers studied the variability in gene expression levels in synovial tissues within and between patients [48]. This study demonstrated that different arthroscopic biopsies taken from one joint result in gene expression signatures that are more similar within the joint of one patient than between patients.

A large-scale gene expression profiling study of synovial tissues from patients with erosive RA revealed considerable heterogeneity among different patients [49,50]. A systematic characterization of the differentially expressed genes highlighted the existence of at least two molecularly distinct forms of RA tissues (**Figure 1**, **Table 1**). One group revealed an abundant expression of clusters of genes indicative of ongoing inflammation and the involvement of the adaptive immune response. This subgroup is referred to as the RA high-inflammation group. The increased expression of Ig genes was shown to be one of the main discriminators between high-and low-inflammatory tissues. Further analyses of the genes involved in the high-inflammation tissues provided evidence for a prominent role of the genes indicative of an activated signal transducer and activator of transcription (STAT)-1 pathway. These findings were confirmed at the protein level [51,52].

The expression profiles of the second group of RA tissues were reminiscent of those of tissues from patients with OA. These profiles revealed a low-inflammatory gene expression signature and an increased expression of the genes involved in tissue remodeling activity, which is associated with fibroblast dedifferentiation. In contrast to the high-inflammation tissues, these tissues had increased levels of matrix metalloproteinase (MMP)11 and 13 expression and a low expression of MMP1 and 3 [49].

Additional histological analysis revealed that tissues that contain GC-like structures were selectively found among the high-inflammation tissues. The gene expression signature of these tissues revealed an increased expression of

Table 1. Overview of genomic and proteomic studies to demonstrate heterogeneity in rheumatoid arthritis.

Source: serum/cell/ tissue type	Number of patients	Procedure: MS/microarray	Relation to clinical parameters	Biological process involved	Ref.
PBMC	19 RA	4300 genes	Early vs established RA (11 vs 8)	Immune/growth factor activity Proliferation/neoplasia	[63]
Whole blood	25 RA	18,000 genes	anti-CCP	Inflammation/immune activity	Van der Pouw Kraan *et al.* in prep.
Plasma	41 early RA 23 early UA	Bio-Plex cytokine array system	anti-CPP and RF	Both pro- and anti-inflammatory cytokines	[42]
Serum	10 RA	MS	Erosive vs nonerosive (5 vs 5)	Inflammation (e.g., CRP and S100 family members)	[36,41]
Serum	18 RA	Synovial proteome arrays; 225 peptides	Shared epitope, RF^+ vs RF^-, CRP levels, disease duration and severity	Antibodies to citrullinated (severe/late) and native autoantigens (mild/early)	[33]
Synovial tissue	23 RA	11,500 and 18,000 genes	ESR	Adaptive immunity (T and B cells and APC), ectopic lymph nodes, STAT-1 pathway, tissue remodeling	[49,50]
Synovial tissue	12 early RA 4 late RA	23,040 cDNA's	Unknown	Synovitis; accumulation of lymphocytes and plasma cells	[53]
FLS	19 RA	18,000 genes	Unknown	TGF-β/activin A pathway, myofibroblast differentiation, IGF2/IGFBP5	[59]

1D: 1-dimensional; APC: Antigen-presenting cells; CCP: Cyclic citrullinated peptide; cDNA: complementary DNA; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; FLS: Fibroblast-like synoviocytes; IGF: Insulin-like growth factor; IGFBP: Insulin-like growth factor binding protein; MALDI-TOF: Matrix-assisted laser desorption ionization time-of-flight; MS: Mass spectrometry; PBMC: Peripheral blood mononuclear cells; RA: Rheumatoid arthritis; RF: Rheumatoid factor; STAT: Signal transducer and activator of transcription; TGF: Transforming growth factor; UA: Undifferentiated arthritis.

approximately 400 genes that reflect the activation of distinct processes that allow attraction, retention and survival of mononuclear cells [Baltus B *et al.* Unpublished Observations]. By contrast, tissues with a diffuse type of infiltrate showed a profile that indicated repression of angiogenesis and increased extracellular matrix remodeling.

Tsubaki and colleagues demonstrated that tissue heterogeneity within RA can already be observed in the early phase of RA [54]. In this study, gene expression profiles were analyzed from synovial lining tissues from 12 patients with early RA (i.e., a duration of less than 1 year after diagnosis) and four patients with long-standing RA (i.e., a duration of more than 3 years after diagnosis). These authors analyzed gene expression signatures of synovial lining tissues selected by laser capture microscopy. As seen in the previous study using tissues from long-standing RA patients, the early RA patients could be divided into at least two different groups based on their gene expression profiles.

Heterogeneity in rheumatoid fibroblast-like synoviocytes

Fibroblasts are ubiquitous mesenchymal cells that play important roles in organ development, inflammation, wound healing, fibrosis and pathology [53]. In chronic inflammation, fibroblasts are considered as sentinel cells that contribute to leukocyte migration and local immune response through the production of various immune modulators [55]. These observations suggest that fibroblasts may acquire the capacity to modulate the immune response [56,57].

One of the first gene expression profile analyses of fibroblast-like synoviocytes (FLS) revealed the overexpression of genes responsible for the tumor-like growth of rheumatoid synovium [58]. In this study, a commercial cDNA array membrane containing 588 cDNA fragments of known cancer-related genes was used to compare the gene expression profile of five RA FLS with five traumatic control patients. An increased expression level was found for platelet-derived growth factor receptor (PDGFR)α, plasminogen-activator inhibitor (PAI)-1 and stromal cell-derived factor 1 α (SDF1A) in FLS derived from rheumatoid synovium compared with normal FLS. Since the sample size in this study was very small, heterogeneity between FLS derived from different RA patients was not considered.

Recently, the authors profiled FLS derived from 19 RA patients using 24K cDNA microarrays [59]. Interestingly, an unsupervised hierarchical cluster analysis of gene expression profiles from the FLS that were cultured from rheumatoid synovium clearly revealed the existence of at least two major subtypes of FLS. Correlation studies of paired synovial tissue and FLS clustering revealed that the heterogeneity at the synovial tissue level is associated with a specific phenotypic characteristic of the cultured resident FLS. Increased production of insulin-like growth factor (IGF)2 and IGF binding protein (BP)5 appears to constitute a characteristic feature of FLS derived from low-inflammation tissues.

The high-inflammation tissues were associated with an FLS subtype that reveals a similarity with so-called myofibroblasts. The myofibroblast is a specialized fibroblast that has acquired the capacity to express α-smooth-muscle actin (SMA), an actin isoform typical of vascular smooth muscle cells. It is now well accepted that the myofibroblast is a key cell for connective tissue remodeling and contributes to cell infiltration. These cells are characterized by a marked increase in the expression of genes that represent the transforming growth factor (TGF)β response program. Among these response genes were SMA, serine peptidase inhibitor (SERPINE)1, Type IV collagen-α chain (COL4A1), immediate early response (IER)3, transgelin (TAGLN) and the gene for activin A as potential agonists for the induction of the TGFβ response program. The molecular feature that defines the myofibroblast-like phenotype is reflected by an increased proportion of myofibroblast-like cells in the FLS population. In addition, myofibroblast-like cells are also identified in RA synovial tissues upon immunohistochemical analysis [59]. Moreover, similar cells have recently been identified in the human(h)TNF$^{+/-}$ transgenic mouse model of arthritis [60]. Oncology studies indicate that myofibroblasts present in tumors play a crucial role in angiogenesis through the production of extracellular matrix proteins, chemokines and growth factors. Hence, it is hypothesized that myofibroblast-like synoviocytes in RA synovial tissue contributes to angiogenesis.

These data support the notion that heterogeneity between synovial tissues is reflected in the FLS as a stable trait and provide evidence for a link between an increased myofibroblast-like phenotype and high-inflammatory synovitis. However, these data need to be confirmed in a separate study.

Heterogeneity in peripheral blood cells

Since RA is a systemic disease, several investigators study gene expression levels in peripheral blood cells to address the question of whether disease characteristics are detectable from gene expression levels in these blood cells (**Figure 1**, **Table 1**). Bovin and colleagues studied the gene expression profiles of peripheral blood mononuclear cells (PBMC) between RA patients (n = 14) and healthy controls (n = 7) using DNA microarrays. Using two independent mathematical methods, genes were selected that discriminate between RA patients and healthy controls [61]. These genes reflected changes in the immune/inflammatory responses in RA patients. Among these were genes for the calcium-binding proteins S100A8 and S100A12, which is in line with the previously described serum protein results. Subsequent analysis to identify differences between RF-positive and -negative RA patients yielded no differences in this study [61]. Batliwalla and colleagues studied gene expression differences between PBMC from RA patients (n = 29) and healthy controls (n = 21). They identified glutaminyl cyclase, IL1RA, S100A12 and Grb2-associated binding protein (GAB2) as the main discriminators [62]. However, these investigators did not address heterogeneity in phenobarbital (PB) gene expression profiles among patients in RA.

Recently, the authors performed gene expression profiling analysis on whole blood cells, which revealed clear and significant differences between RA patients (n = 35) and healthy individuals (n = 15). The microarray data confirmed previous observations of increased expression of the calcium-binding proteins S100A8 and S100A12. The application of gene set analysis algorithms to identify gene sets that reflect a distinct pathway or biological process revealed an increased expression of immune defense genes, including Type I IFN-response genes, suggesting that this pathway is activated systemically. Interestingly, the increased expression of immune defense genes was characteristic of not all, but approximately half of the patients. Moreover, the authors found that the subset of patients with an increased immune defense profile have significantly increased titers of anti-CCP. The authors concluded that the activation of an immune response among the gene sets with a Type I IFN signature defines a subgroup of RA patients characterized by an increased autoreactivity against citrullinated proteins.

Olsen and colleagues studied gene expression levels in PBMC in order to identify differentially expressed genes between early (a disease duration less than 2 years) and established RA (an average disease duration of 10 years) [63]. Out of 4300 genes analyzed, nine were expressed at threefold higher levels and 44 genes were expressed at threefold lower levels in the early RA group compared with the late RA patients. The genes expressed at higher levels in early RA include CSF3 receptor, cleavage stimulation factor and TGF-β receptor II, which affect B-cell function. Genes involved in immune/inflammatory processes and genes related to cell proliferation and neoplasia were expressed at lower levels in early arthritis. Interestingly, approximately a quarter of at least twofold upregulated early arthritis genes overlapped with an influenza-induced gene set. This finding led the authors to suggest that the early arthritis signature may partly reflect the response to an unknown infectious agent.

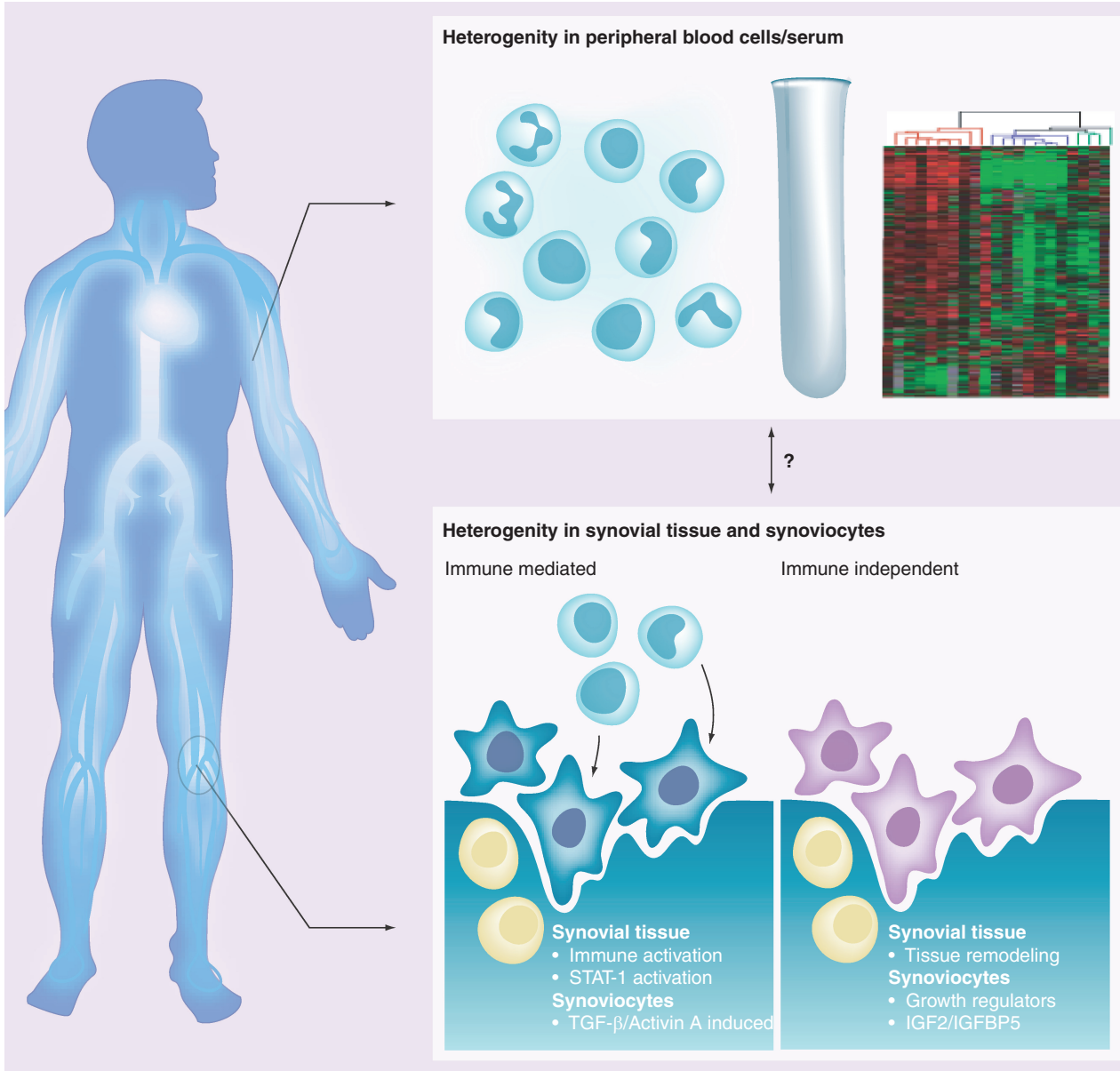
Conclusions

Gene expression profiling combined with proteomics technology provides an opportunity for a system biology approach to understanding disease. Exploratory research activities in this field have powered disease subclassification and support the existence of distinct processes that contribute to disease and tissue destruction in RA (**Figure 2**). A critical question to answer is how these data relate to clinical parameters. The challenge we face is how to integrate the information on molecular profiling in RA with clinical parameters in such a way that it can be applied in a clinical setting to improve patient stratification and decision making in clinical practice. The authors postulate that this type of research will not only amplify our knowledge of the disease but also provide the foundation for individualized therapies targeting deregulated pathways that drive key biological processes contributing to the disease. Hence, future therapies in RA will be tailored to the unique biology of an individual patient.

Future perspective

Evidence is accumulating that large-scale gene and protein expression profiling will contribute considerably to our understanding of the molecular and biological basis of the well recognized, but as yet poorly defined, heterogeneity of RA. The available data from genomics and proteomics studies in RA indicate that the observed molecular heterogeneity

Figure 2. Discovery of rheumatoid arthritis subtypes in peripheral blood cells and synovial tissue.



A schematic overview of the discovery of RA subtypes in peripheral blood cells and synovial tissue. Heterogeneity in RA can be shown on a peripheral level using peripheral blood cells or serum. Using high-throughput molecular analyses, patients can be subclassified on the basis of their gene expression profile in peripheral blood cells, or on their protein profile in sera.
Hierarchical clustering of differentially expressed genes in peripheral blood cells from RA patients has highlighted the existence of at least two molecular distinct forms of RA (see dendogram at the top of the cluster diagram). This subclassification of RA patients was also observed in synovial tissues and synoviocytes. It is hypothesized that two different pathogenic mechanisms are playing a role in RA. The first is an immune-mediated destruction of cartilage via activation of STAT-1 and TGF-β programs. The second is an immune-independent mechanism, possiblly mediated by tissue remodeling and growth factors. The relationship between the blood and tissue profile needs further investigation.
IGF: Insulin-like growth factor; IGFBP: IGF binding protein; RA: Rheumatoid arthritis; STAT: Signal transducer and activator of transcription; TGF: Transforming growth factor.

in RA mainly reflect differences in immune and inflammation activity (**Table 1**). The existence of such molecular heterogeneity in rheumatoid synovial tissues and cells fits a model proposed by Firestein and Zvaifler, who suggested two independent processes [64]: an immune-mediated and a stromal cell-driven form, which drive destruction of bone and

cartilage. We postulate that this type of research will amplify our understanding of disease subtypes and underlying biology. These developments open the way for a 'redefinition' of RA.

The molecular markers (biomarkers) that distinguish patients from one another can be used for diagnosis (early detection), prognosis (predicting outcome), determining treatment efficacy (translational research) and risk management. The challenge we face is to integrate the information on differential gene expression and protein patterns in RA with clinical parameters in such a way that it can be applied in a clinical setting to improve patient stratification and decision making in clinical practice. The transition of this explorative type of research to integrated clinical diagnostics will require extensive optimization of the technological procedures. Moreover, the assignment

Executive summary

Rheumatoid arthritis is a heterogeneous disease

- Rheumatoid arthritis (RA) patients reveal a striking heterogeneity based on clinical, biological and molecular criteria. The categorization of patients is expected to be of utmost importance for decision making in clinical practice. Recent developments in high-throughput screening technologies have provided the opportunity to characterize patients based on their molecular profile. The identification of differentially expressed genes and proteins may provide a comprehensive molecular description of disease heterogeneity that will probably uncover biomarkers to allow the stratification of RA patients.
- When associated with clinical readouts, the clinical useful molecular markers could be selected and applied in day-to-day clinical practice.

Autoantibodies in RA

- The presence of specific autoantibodies against antigens containing one or more citrulline residues, the so called anticyclic citrulline peptide (CCP) antibodies and rheumatoid factor (RF), are instrumental in classifying the diagnosis. These autoantibodies are already present before the appearance of clinical signs of arthritis. The production of these autoantibodies is an early process and their appearance is predictive of the development of RA.
- The presence of anti-CCP in sera from RA patients appears to identify a subgroup of RA patients with an increased chance of developing persistent and erosive disease. A combination of biomarkers, which may include RF and anti-CCP, could reflect different aspects of the disease process and therefore might be useful for evaluating prognosis in individual patients with early RA.

Protein markers in RA

- Emerging developments in proteomics technologies hold the promise to contribute to better diagnostics and prognostics, and disease management. Increased levels of S100 proteins, in combination with C-reactive protein in sera of RA patients are associated with erosive disease. Multiplex analysis of cytokines identified composite profiles that are associated with 'mild' and 'severe' forms of RA. Further developments in multiplex protein measurements have profound potential for the future.

Heterogeneity in RA tissues revealed by large-scale gene expression profiling

- DNA microarray technology provides a powerful way to gain insight into the molecular complexity of cells and tissues and allows an open-ended survey to comprehensively identify the fraction of genes that are differentially expressed among patients with RA. Large-scale gene expression profiling of synovial tissues has revealed considerable heterogeneity among different patients with RA. At least two molecularly distinct forms of RA tissues were identified. These differences can be observed in the early phase of RA. These findings clearly support the existence of disease subtypes and provide an insight into the distinct processes that contribute to disease and tissue destruction in RA.

Heterogeneity in rheumatoid fibroblast-like synoviocytes

- Heterogeneity between synovial tissues is reflected in the fibroblast-like synoviocytes as a stable trait and provides evidence for a link between an increased myofibroblast-like phenotype and high-inflammatory synovitis.

Heterogeneity in peripheral blood cells

- The activation of an immune defense response, including genes that represent a Type I interferon response program, in the peripheral blood cells of RA patients defines a subgroup of patients characterized by increased titers of anti-CCP. This immune defense response gene signature appears to exist in early RA and possibly reflects the response to an unknown infectious agent.

Future perspective

- These developments open the way for a 'redefinition' of RA. The authors postulate that a detailed molecular description of the disease will not only amplify our knowledge of the disease and its subtypes, but will provide the foundation for individualized therapies targeting deregulated pathways that drive key biological processes contributing to the disease. Future therapies in RA will be tailored to the unique biology of an individual patient.

and implementation of useful and reliable classifiers requires rigorous standardization and several levels of validation.

It is anticipated that molecular profiling of RA will contribute considerably to explaining the heterogeneity in treatment response. Future treatment will be tailored to the molecular and biological features of an individual patient. This will lead to personalized medicine, in other words the prescription of therapeutics that are best suited to each individual patient.

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