3

Gene Expression Profiling in Rheumatoid Arthritis

Cornelis L. Verweij and Saskia Vosslamber
Department of Pathology and Rheumatology; VU University Medical Center, Amsterdam, The Netherlands

1. Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease that primarily affects the joints. Aetiology of RA is unknown. Once symptoms are present RA manifests itself as a heterogeneous disease with a clinical spectrum ranging from mild to severe disease, and variable involvement in secondary organ systems. The heterogeneous nature is reflected by variation in responsiveness to treatment. The heterogeneity most likely has its origin in its multifactorial nature, whereby specific combinations of environmental factor(s) and genetic factors are likely to influence not only susceptibility but also the disease severity and prognosis. Unfortunately, our understanding of the molecular complexity of RA is incomplete, and criteria for subtyping of patients, e.g. for prognosis to select those patients who will benefit from a specific treatment, are currently lacking.

By definition, nearly every aspect of a disease phenotype should be represented in the pattern of active genes and subsequent transcripts and proteins that are expressed. DNA microarray technology is a powerful technique that enables studying of mRNA levels of all the genes in the genome simultaneously. Application of large-scale gene expression profiling using DNA micro arrays (genomics) of blood and tissue samples from patients with RA allows an open-ended survey to identify comprehensively the fraction of active genes that are specific for a clinical condition (figure 1). This information provides insight in biological pathways contributing to disease and to identify classifiers for early diagnosis, prognosis, and response prediction.

Due to the complexity of the microarray technology and sometimes not optimal powered studies, and high costs associated with use of this technology, several important aspects need to be considered when analyzing micro array data. In almost all cases, the number of transcripts that is measured on an array is much higher than the number of samples included in a study and therefore there is a high change of 'false positives' of which one should be aware and account for in the analysis. Thus, good laboratory proficiency for data acquisition needs to be ensured and appropriate and properly used data analyses practices are essential.

Initially several pitfalls were experienced using this multistage technology. Factors that could influence the sensitivity and reproducibility range from differences in sample storage and processing, variation in amount and quality of starting RNA, RNA amplification and

labeling strategies, solid-phase DNA sequences and hybridization conditions. In addition the lack of standardized approaches for normalization and usage of data analysis algorithms could influence the outcome. Hence, the application of perfectly standardized conditions is crucial to generate high quality data points. Moreover, verification of results became an essential step in microarray studies. In order to set quality criteria for performing and publishing microarray studies, standards for microarray experiments and data analysis were created (Brazma et al., 2001).

Cluster algorithms are very useful in visualizing huge datasets obtained with microarray experiments. Data can be clustered according a predetermined separation of the patient samples (supervised), or driven by molecular variation (unsupervised). Very often the data set becomes more comprehensive by selecting only genes that are differentially expressed between groups of patients/samples. Filtering of expression data by applying a threshold for a certain fold change compared to the median expression levels in at least a certain number of patients studied results in a condense and informative set of differentially expressed genes. Additional, pathway-level analyses, and classifier and prediction algorithms can provide more insight in the functional pathways or biological processes and markers for stratification, prognosis and response prediction.

Now, after years of technical and analytical improvement, the technology and algorithms for data analysis are robust and reproducible across properly designed and controlled experiments between different research groups. In addition the introduction of PAXgene (PreAnalytix, GmbH, Germany) whole blood aspiration system, whereby cells are directly lysed and the RNA stabilized, excludes *ex vivo* processing artifacts and forms an essential step in the standardization process. However, careful standardization is still required for cell subsets and tissues that are obtained via *ex vivo* manipulation.

This review describes developments in transcriptomics research to identify novel pathways that contribute to disease and to uncover clinically relevant biomarkers. Ultimately this information may help clinicians to improve disease management.

2. Gene expression profiling in affected target tissues and cells

2.1 The rheumatoid synovium

Since synovitis is the hallmark of rheumatoid arthritis, gene expression analysis was initially aimed to provide insight in the molecular features and biological pathways at play in the affected synovium. The first study on gene expression profiling in rheumatoid synovium highlighted the increased expression of genes involved in chronic inflammation such as immunoglobulins and HLA-DR in RA synovium when compared with normal synovium (Zanders et al., 2000) Comparative analysis of synovial tissue specimen from RA and osteoarthritis (OA) patients revealed that these diseases were characterized by distinct synovial gene signatures (van der Pouw Kraan et al., 2003a; van der Pouw Kraan et al., 2003b; Sha et al. 2003; Devauchelle et al., 2004; Kato et al. 2007; Nzeusseu et al. 2007; Huber et al. 2008). The finding that genes involved in the adaptive immunity (B and T cell regulation) were upregulated in RA tissues confirmed histological findings of increased infiltration of T cells and B cells in the rheumatoid synovium compared to OA. In addition, a number of non-immune genes were found to be differentially expressed between the RA and OA synovium, which were involved in diverse biological processes such as extracellular

matrix biology (e.g. fibronectin, fibulin-3 and collagen type IIIa1), transcription and cell cycle regulation (CAK, DNA replication licensing factor, CDK7, FOS, CHD2), receptor/signaling (GBP1 IL1R1, CXCL2, PDGFRA), protease biology (Cathepsin L and Cathepsin D, adhesion paxillin, integrina2, D66) and apoptosis (BECN1). Analysis of 3265 genes led to the discovery of a 21 gene discriminator between RA and OA synovial tissue (Kato et al. 2007). Devauchelle found a 48 gene discriminator out of 5670 genes studied (Devauchelle et al. 2004). Using an array with 1050 gene sequences with a combination of a binary probit model with bayesian variable selection, Sha and colleagues found several small gene sets that led to good classification results. A similar study was performed by Huber and colleagues (Huber et al. 2008) who identified three pathways with significantly higher variances in RA (e.g. B-cell receptor signaling and vascular endothelial growth factor signaling) compared to OA. Functionally, the majority of the identified pathways are involved in the regulation of inflammation, proliferation, cell survival and angiogenesis.

Additional comparative analyses of synovial biopsy tissue from patients with RA, OA and systemic lupus erythematosus (SLE) confirmed and extended observations that distinct diseases were characterized by distinct molecular synovial signatures (Nzeusseu et al. 2007). Overall, tissue profiling in RA and other rheumatic diseases has led to an increase in our understanding of disease pathogenesis. These findings highlight the molecular differences between the RA, OA and SLE synovia and demonstrate that transcriptome analysis provide a rich source for the establishment of diagnostic tools and may lead to identification of novel drug targets.

2.1.1 Heterogeneity between rheumatoid synovial tissues

The lack of a consistent comprehensive transcript profile in RA synovium may be due to the small samples sizes, heterogeneity between disease tissues, differences in appropriate control tissues, and/or technical differences such as the variation in the type and the complexity of the arrays used by the different research groups.

A large-scale gene expression profiling study of 30 synovial tissue specimens from patients with erosive RA revealed considerable heterogeneity among patients (van der Pouw Kraan et al., 2003a; van der Pouw Kraan et al., 2003b). Also Huber and colleagues noted the broad intra-group inter-individual expression variances in RA for genes representing different pathways (e.g. Toll-like receptor signaling pathway, T-cell receptor signaling pathway, Fc epsilon receptor I signaling pathway, adherence junction, classical TGF-β sub-pathway and the anti-apoptotic sub-complex (Huber et al., 2008). Accordingly, Lindberg and colleagues showed that synovial biopsies had gene expression signatures that were unique for each patient (Lindberg et al., 2006a). Heterogeneity is not surprising known the variation in clinical presentation, differences in treatment outcome and complex pathogenesis that changes over time.

Systematic characterization of the differentially expressed genes highlighted the existence of at least two molecularly distinct forms of RA tissues (van der Pouw Kraan et al., 2003a; 2003b). One group, referred to as the RA high inflammation group, was characterized by genes involved in inflammation and adaptive immune response. The genes involved in the high inflammation tissues consist of immunoglobulin genes and genes indicative for an activated IFN/STAT-1 pathway. Seven of these (TIMP2, PDGFRA, GBP1, Fos, CTSL, TUBB

and BHLHB2) were also described by Devauchelle and colleagues, of which 2 (GBP1 and CTSL) are known to be regulated by type I IFN (Devauchelle et al., 2004). The second group of RA tissues was characterized by a low inflammation gene signature that was reminiscent of that of tissues from patients with OA. While inflammation and immune-related genes were decreased, these tissues showed an increased expression of genes involved in tissue remodeling activity, which is associated with fibroblast dedifferentiation. Remarkably, the high and low inflammation tissues revealed reciprocal expression of specific matrix metalloproteinases (MMP). Whereas levels of MMP11 and 13 were increased in low inflammation tissues, levels of MMP1 and 3 were increased in high inflammation tissues (van der Pouw Kraan et al., 2003a).

Histological analyses already revealed the existence of different tissue types in the rheumatoid synovium that are related to differences in the cell distribution (Takemura et al., 2001). In approximately 10% of synovial tissues T cells, B cells, and follicular dendritic cells (FDCs) are organized into germinal centres (GC) like structures. The other tissue types lack FDCs and show either a diffuse or an aggregated T-cell and B-cell infiltrate.

The tissues with these so called ectopic GCs were selectively present in the high inflammation tissues. These tissues revealed increased Ig transcript expression with the concomitant presence of B cells and/or plasma cells, which may support local production of antibodies. Gene expression revealed concomitant expression of genes encoding the chemokines CXCL12 and CCL19 and the associated receptors CXCR4 and CXCR5, which are important for the attraction of T cells, B cells, and dendritic cells, in GC containing tissues (Timmer et al., 2007). In addition genes involved in T-cell and B-cell specific pathways, and Fc-receptor type I and JAK/STAT signaling. Elevated expression of IL-7 receptor α(IL-7Rα)/IL-2Rγ chains and IL-7) suggest a role for the IL-7 pathway in synovial lymphoid neogenesis in RA. Tissues with a diffuse type of infiltrate exert evidence of repressed angiogenesis and increased extracellular matrix remodeling.

Overall, the gene expression profiling of rheumatoid synovium has provided insight into the molecular basis of the heterogeneous nature of synovial disease pathogenesis in RA and may facilitate subclassification of patients based on a synovial marker profile *(figure 1).* However, it remains to be determined if a specific molecular profile applies to all affected synovia in a single patient, and if the profile is stable during the course of disease.

2.2 Gene expression in mesenchymal cells derived from affected target tissues

Fibroblast-like synoviocytes (FLS) are major players in joint destruction in RA. FLS have a transformed phenotype and act as sentinel cells that contribute to leucocyte migration and local immune response through the production of various immune modulators (Smith et al., 1997; Hogaboam et al., 1998; Brouty-Bové et al. 2000). Reversibly, the soluble factors, such as cytokines and growth factors released from the immune cells, in combination with cell-cell interactions likely activate FLS and influence their behavior. One of the first gene expression analyses of in-vitro cultured FLS clearly demonstrated over-expression of genes responsible for tumor-like growth (Watanabe et al., 2002). Analysis of the expression of 588 known cancer-related genes revealed increased expression of PDGFRα, PAI-1 and SDF1A by FLS from five patients with RA compared to FLS from five traumatic control patients. Galligan and colleagues performed a comparative gene expression analysis on FLS cultured from RA

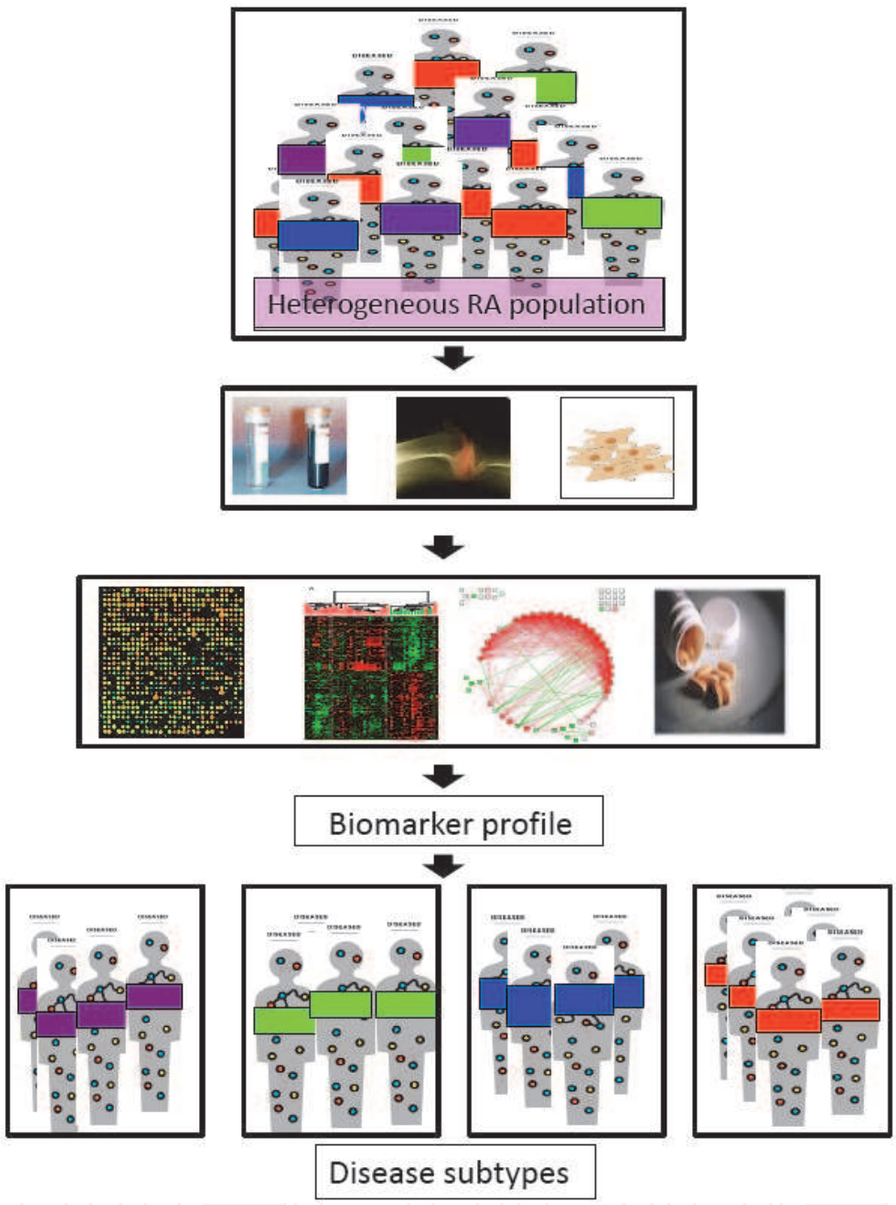


Fig. 1. Schematic outline for disease subclassification in RA

Legend: RA patients reveal a striking heterogeneity based on clinical, biological and molecular criteria. Categorization of patients is crucial for decision making in clinical practice. Recent developments in high-throughput screening technologies makes it possible to characterize patients based on their molecular profile. Application of DNA-microarrays enables the generation of a transcript profile (barcode) of an individual patient. When associated with clinical read-outs clinical useful molecular markers could be selected and applied in day-to-day clinical practice. The procedure starts with collecting biosamples (e.g. peripheral blood cells) from each patient. The biosample can be processed to isolate mRNA and then further analyzed using DNA-microarray technology. Subsequently, computational algorithms will be applied to select biomarkers that allow subtyping of patients. This approach helps to elucidate the distinct pathological mechanisms that can explain the inter-patient variation, disease progression, and treatment response.

(17), OA (20) and trauma (6) joint tissue using affymetrix microarrays (Galligan et al., 2007). A total of 34 genes were significant differentially expressed between RA and OA FLS. Genes highly and exclusively expressed by RA FLS are HOXD10, HOXD11, HOXD13, CCL8 and LIM homeobox 2. Genes encoding CLU, sarcoglycan-y, GPR64, POU3F3, peroxisome proliferative activated receptor-y and tripartite motif-containing 2 were exclusively expressed in OA FLS. Interestingly, only a few of the significant differently expressed in FLS also differed between the total synovial tissue expression profiles, suggesting that the contribution of the synovial lining cells is not dominant in the total tissue profile. Alternatively, the transcriptome of in-vitro cultured FLS may not be representative for the genuine transcriptome of in situ FLS. Evidence that in vitro cultured FLS gene expression changes are beyond the 10% when passaged less than 6 times, as was done by Galligan and colleagues, suggest that this was not the case (Nuemann et al., 2010). However, formal proof by comparison of in-vitro cultured FLS with in-situ FLS (via e.g. Laser Capture Microscopy derived cells) is lacking.

Others investigated the effects of tumour necrosis factor (TNF) and IL-1b on FLS. TNF and IL-1b have been shown to be of primary importance in the effector phase of the disease. Defining TNF-α and IL-1b response signatures in FLS may be instrumental for application in pharmacology studies to monitor the effects of TNF and IL-1b blockade. In an early microarray study 96 inflammatory genes were studied in cytokine-stimulated RA-FLS cells. A number of cytokine-regulated genes such as IL-6, CXCL8, CXCL1, MMP-1, MMP-3, MMP-8 and VCAM-1 were identified (Heller et al., 1997). Additional studies using arrays with higher complexity (12.600 genes) revealed that TNF affected the expression of genes representing cytokines and inflammatory mediators, extracellular matrix and adhesion molecules, cell cycle and proliferation related proteins, transcription related proteins, and apoptotic mediators (Gallagher et al., 2003). One of these TNF-response genes, Nef-associated factor-1 (Naf1, an A20-binding, nuclear factor kappa B (NFkB) inhibitory protein), was identified as indicator for TNF-bioactivity. This analysis revealed higher expression in synovial biopsies from patients with active RA and seronegative arthropathy than in those from patients with OA. Taberner and colleagues showed that there exists a broad overlap between TNF- and IL-1b response genes. Out of 12600 genes tested 126 genes were regulated by both TNF and IL-1b, 65 genes were specifically regulated by IL-1b (e.g. G-CSF, CXCL6, CCL4, PAI-1, OAS 40kD and VEGF) and 21 genes by TNF (e.g. CXCL19, CXCL11, PIAS3, ID1, MAPKKK4) (Taberner et al., 2005). It is likely that these response signatures contain numerous genes that contribute critically to the pathogenesis of RA and provide a framework to unravel IL-1b and TNF driven effector pathways.

Detailed analysis of the gene expression profiles clearly revealed that the profiles are not uniform between RA patients, analogous to the gene expression of the synovial tissues. Transcriptome analysis of FLSs derived from 19 RA patients using microarrays with a complexity of 24,000 cDNA elements, revealed 3 molecular profiles, indicative for the existence of 3 FLS subtypes (Kasperkovitz et al., 2005). Accordingly, others also noted marked heterogeneity in both the gene expression profile (Galligan et al. 2004) as well as the sensitivity to proinflammatory cytokines between FLS derived from different RA patients (Taberner et al. 2007). Correlation studies of paired synovial tissue and FLS clustering revealed that heterogeneity at the synovial tissue level is associated with a specific phenotypic characteristic of the cultured resident FLS (Kaperkovitz et al., 2005). The high

inflammation tissues were associated with an FLS subtype that exhibits similarity with so-called myofibroblasts. The myofibroblast is a specialized fibroblast that has acquired the capacity to express α-smooth muscle actin, an actin isoform that is typical of vascular smooth muscle cells. The myofibroblast is a specialized fibroblast that plays a key role in connective tissue remodelling and contributes to cell infiltration. These cells are characterized by a markedly increased expression of genes that represent the transforming growth factor (TGF)-β response programme. Among these response genes were SMA, SERPINE1, COL4A1 (type IV collagen-α chain), IER3 (immediate early response 3), TAGLN (transgelin) and the gene encoding activin A, which is a potential agonist for the induction of the TGF-β response programme. Similar cells were recently identified in the human $TNF^{+/-}$transgenic mouse model of arthritis (Aidinis et al., 2005). Moreover, Pohlers and colleagues noted constitutive activation of the TGF-β pathway in RA FLS (Pohlers et al., 2007). A significant positive correlation were observed between the constitutive expression of TGF-β1 mRNA (but not protein) and the serum levels of C-reactive protein was observed. Myofibroblasts may play a crucial role in angiogenesis through the production of extracellular matrix proteins, chemokines and growth factors, as has been shown in the field of oncology. Hence, it is proposed that these cells contribute to angiogenesis in the rheumatoid synovium. This finding supports the hypothesis that phenotypic variation between FLS may be causally related to the inflammation status of the target tissue.

2.3 Gene expression in rheumatoid bone marrow

Evidence exists that bone marrow-derived mononuclear cells (BMMC) contribute to the pathogenesis of RA (Ochi et al., 1988; Jongen-Lavrencic et al., 1997; Hirohata et al., 2004). The bone marrow harbors three types of stem cells: the mesenchymal stem cells, the hematopoietic stem cells and the endothelial stem cells. The local cell-cell interactions and soluble factors act in a sophisticated network that regulates the proliferation and differentiation of these cells. Elevated levels of IL-6 and IL-8 in RA bone marrow serum was reported to be associated with synovial hyperplasia (Tanabe et al., 1994). Comparative gene expression profiling between RA and OA BMMC revealed marked variation between the two diseases (Nakamura et al., 2006; Lee et al., 2011). Transcriptome analysis identified 2,674 genes which were differentially expressed between RA and OA BMMC (Lee et al., 2011). Marked upregulated genes were classified as immune response genes, which were highly relevant to the antigen presentation pathway (e.g. HLA-E, HLA-F, HLA-G, tapasin (TAP), TAP binding protein) and interferon (IFN) signaling (e.g. IFITM1, IFITM3, IFI16, MAPK14, MyD88, IL8). In a third network IFNɣ played a central role (e.g. PSM8, PSM9, CLEC5A, CLEC4E). A fourth network was centered around HNF4 and involved in lipid metabolism, coagulation and negative regulation of cell growth. The downregulated genes were dominantly related to cell cycle and DNA metabolism. These findings provide important information to abnormal BMMC biology in RA. It remains to be determined why these processes are disturbed and how these abnormalities contribute to the pathogenesis of RA.

2.4 Gene expression in blood cells

Known the systemic nature of RA and the communication between the systemic and organ specific compartments, whole blood and/or peripheral blood mononuclear cells (PBMC) is a

useful compartment to study the disease-related gene expression profiles. Because of the low-invasiveness blood aspiration this compartment is extremely suitable for explorative studies in large cohorts of patients to identify clinically relevant biomarkers.

Several investigators studied gene expression levels in peripheral blood cells to address the question, whether disease specific features were present in peripheral blood cells. Bovin and colleagues identified 25 genes immune related genes (e.g. calcium-binding proteins S100A8 and S100A12) that discriminated between PBMC of RA patients (n=14) and healthy controls (n=7) (Bovin et al., 2004). S100A8 and S100A12 (calgranulins C) belong to a class of inflammatory mediators, and function as heterodimers. These proteins are released by e.g. activated monocytes upon interaction with activated endothelial cells under inflammatory conditions and mediate leukocyte migration and adhesion to vascular endothelium. Liao et al. used tandem mass spectrometry (MS/MS), coupled with multidimensional liquid chromatography (LC) to identify biomarkers of disease severity in the synovial fluid and serum of patients with RA (Liao et al., 2004). Levels of CRP, S100A8, S100A9 and S100A12 were elevated in the serum of patients with erosive disease compared with patients with non-erosive RA. No significant differences between RF positive and RF negative RA were observed. In a larger study with 29 RA patients and 21 healthy controls Batliwalla and colleagues identified 81 differentially expressed genes (e.g glutaminyl cyclase, IL1RA, S100A12 and Grb2-associated binding protein (GAB2) as the main discriminators. This profile correlated with an increased monocyte count (Batliwalla et al., 2005). Studies with a preselected set of 96 genes in PBMC from IBD, psoriasis and RA patients, and healthy controls revealed genes (e.g. ADM, AQQ9, CXCL12, IL10, NAMPT) that were specific for the chronic inflammatory diseases in general, and disease specific genes (Mesko et al., 2010). Genes that were specific for RA included mainly downregulated genes e.g CCL4, CCL5, CDNK1C, CYP51A1, FGL2, HMGB1, IL23R, and PTPN22, and only IL-8 as upregulated gene. Additional studies on peripheral blood cells, including analyses on whole blood cell samples (PAXgene) confirmed and further extended the molecular differences between the peripheral blood compartment between healthy controls and RA patients (van der Pouw Kraan et al., 2007; 2008; Teixeira et al., 2009). Additional genes that Teixeira and colleagues identified include Ly96/MD2, NFAT5, thioredoxin, CAP/LL37, ORM1, ORM2, SLC11A1, PGLyRP1 and Factor V.

Van der Pouw Kraan and colleagues observed that a prominent cluster of IFN-response genes was significantly upregulated in patients with RA indicating that this pathway is systemically activated in RA (van der Pouw Kraan et al., 2007). This cluster contains highly correlated genes such as IFRG28 (28 kDa interferon-responsive protein), IFI35 (interferon-induced protein 35), IFI44L (interferon-induced protein 44-like), IFIT1 (interferon-induced protein with tetratricopeptide repeats 1), IFIT2, IRF2 (interferon-regulatory factor 2), IRF7, GIP2 (interferon a-inducible protein 2), GIP3, SERPING1 (serine proteinase inhibitor clade G member 1, C1 inhibitor), OAS1 (29-59-oligoadenylate synthetase 1), OAS2, MX1 (Myxovirus resistance 1), ISG15 (interferon-induced protein 15) and RSAD2 (radical S-adenosyl methionine domain containing 2). These findings have now been replicated in several other studies using independent cohorts (Thurlings et al., 2010; O'Hanlon et al., 2011; Higgs et al., 2011; Vosslamber et al., 2011). Moreover, IFN-bioactivity was measured in RA serum. Comparative analyses on the extent of the IFN response activity in the blood cells of RA and

SLE patients revealed a 5-fold higher level of expression in SLE compared to RA patients (Higgs et al. 2011, Vosslamber et al., unpublished observation).

2.4.1 Gene expression heterogeneity in blood vs. synovial tissue

Synovial tissue heterogeneity is likely to reflect differences in the underlying disease pathogenesis. Because of the migration of immune cells to and from lesional sites via the blood as well as the recirculation of immune cells between central and peripheral lymphoid organs, the PB compartment could be an easy accessible compartment to monitor the (immune)pathophysiology of the synovial tissue. Hence, a critical question to answer is whether molecular heterogeneity at the synovial tissue level is reflected in the blood. The identification of processes and biomarkers in PB may facilitate informative studies of a relation between tissue type and clinical parameter. The notion that molecular heterogeneity is present in both the synovial as well as the PB compartment makes it tempting to speculate on molecular and biological features that reflect the tissue pathology (van Baarsen et al., 2010b). However, paired analysis of peripheral blood and affected synovium from 17 patients with RA revealed that differential tissue pathology was not reflected in the PB by differential expression of single genes. Pathway-level analysis showed that co-ordinately regulated genes involved in protein synthesis in PB were associated with high-inflammation tissue types. The increased protein synthesis activity in PB could provide a framework for further studies to identify PB biomarkers representative for the tissue inflammation status.

2.4.2 A type I IFN response signature in the peripheral blood of a subset of RA patients

The significantly differential expression of the IFN-response genes indicates that this pathway is activated systemically in RA. Van der Pouw Kraan provided evidence that this signature is specific for type I IFNs (IFNα/β) (van der Pouw Kraan et al., 2006). Thus this type I IFN signature may be a direct reflection of increased type I IFN activity or other ligands known to activate the type I IFN/STAT-1 pathway. The fact that the serum IFN bioactivity could be inhibited by neutralizing antibodies directed against IFNα and IFNβ, provides evidence for a role of both type I IFNs in the induction of IFN type I response activity in RA (Mavragani et al., 2009). Upregulation of type I IFN-response genes has now been observed in peripheral blood cells and/or target tissues of (a subset of) patients with autoimmune diseases such as RA, SLE (Baechler et al. 2005), SSc (Tan et al, 2006; Bos et al., 2009), SS (Mavragani & Crow, 2010), multiple sclerosis (Van Baarsen et al., 2006) and type 1 diabetes. These findings suggest that an activated IFN response gene expression program is a common denominator in chronic inflammatory diseases in general.

Interestingly, the increased expression of the type I IFN response genes was characteristic of not all, but approximately half of the RA patients, consistent with the heterogeneous nature of RA. Moreover, the immune defense gene program that was activated in the subgroup of RA patients was reminiscent to that of virus-infected macaques (van der Pouw Kraan et al., 2008). Comparative analysis between paired tissue and peripheral blood profile revealed that there exists no concordance in the presence of IFN-response activity between the two compartments (van Baarsen et al., 2010a)

2.5 Monozygotic twins and first degree relatives

O'Hanlon and colleagues studied gene expression profiles of monozygotic (MZ) twin pairs discordant for RA, SLE and idiotypic inflammatory myopathies, their unaffected twins and healthy controls (O'Hanlon et al., 2011). Probands differed significantly in gene expression for 92 genes involving several pathways including immune responses, signaling pathways, transcription/translation regulators, and metabolic functions. As part of the immune response genes they observed that IFN-response genes (IFI27, OASF, PLSCR1, EIF2AK2, TNFAIP6, and TNFSF10) were up-regulated in probands compared to unrelated controls. In unaffected twins intermediate ordering was observed for 84 of the 104 transcripts whose expression differed significantly between probands and unrelated controls. Suggesting that unaffected twins may be in a transitional or intermediate state of immune dysregulation between twins with an autoimmune disease and unrelated controls, perhaps predisposing them to the development of systemic autoimmune diseases given the necessary and sufficient environmental exposures. Maas and colleagues, reported similar results when comparing gene expression profiles between PBMCs from patients and unrelated unaffected individuals (Maas et al., 2005). A total of 127 genes was shared between patients with autoimmune diseases and unaffected first-degree relatives. This commonality between affected and unaffected first-degree relatives suggests a genetic basis for these shared gene expression profiles.

2.6 B-lymphocytes

Szodoray and colleagues compared gene expression differences in pooled peripheral blood B cells from 8 RA patients to the pool of B cells from 8 healthy controls (Szodoray et al., 2006). A total 536 genes were differentially expressed between rheumatoid and healthy B-cells (e.g. S100A9, S100A9, CNNM4, BARD1, U5-116KD, TLR9, IL5-RA, IL-10, IL12A, PTX3, CRLF1, CHRNB1, DRD2, MMP28, VEGFC and FOXo3a). These genes were involved in diverse processes including cell–cycle regulation, proliferation, apoptosis, autoimmunity, cytokine networks, angiogenesis and neuron-immune regulation. E.g. the overexpression of FoxO3a in B cells from patients with RA is reported by others and may increased survival of blood PMNs and T lymphocytes. Functional pathway analysis demonstrated that many of these genes were regulated by cytokine and growth factor activity and correlated with significantly increased serum levels of IL-1b, IL-5, IL-6, IL-10, IL-12p40, IL-17 and VEGF, whereas 231 genes were downregulated in RA B cells.

2.7 CD4 T-lymphocytes

Gene expression analysis of CD4 T cells from 21 patients with RA revealed marked heterogeneity between patients reflected by differential expression of 29 genes (including IFI27, Col6A1, RASD1, TLR4, APOA1, SPP1) in processes such as Toll-like receptor signaling pathway, Calcium signaling pathway, cell adhesion molecules, PPAR signaling pathway, and fatty acid metabolism. The differential expression of IFI27 between patients is in line with earlier observations of interindividual differences in IFN type I response activity among RA patients (Chen et al., 2010).

3. Clinical relevant gene signatures

The success of novel insight in molecular patterns and biological processes in disease pathogenesis and the ability to categorize patients based on molecular criteria held out the promise that this approach might yield clinical useful information. Hence, the next research challenge is to use this information on molecular interindividual heterogeneity to the benefit of patients. Research in this field has primarily focused on (very) early diagnosis, prognosis and prediction of therapy responsiveness.

3.1 Genes and signatures involved in disease activity

Molecular heterogeneity at the tissue level can be a consequence of disease stage and duration. Van Baarsen and colleagues studied the synovial tissue gene expression profiles in relation to immunohistochemical scores and disease parameters. The results demonstrated an excellent correlation between molecular and immunohistochemical scoring (van Baarsen et al., 2010b). Moreover, the high inflammation tissue type was predominantly observed in patients with high disease activity and short disease duration, suggesting temporal differences in the inflammatory status during the course of the disease (Firestein & Zvaifler, 2002). These findings support the hypothesis that RA progresses from an inflammatory, and T- and B-cell driven disease to a more immune-independent process that may be driven by ”transformed” FLS (Firestein & Zvaifler, 2002).

The strong association of FLS subtype with synovial tissue inflammation status suggests that FLS markers correlate with disease parameters. Galligan and colleagues showed several significant correlations; (HLA)-DQA2 with HAQ score; Clec12A with RF; MAB21L2, SIAT7E, HAPLN1 and BAIAP2L1 with CRP level; RGMB and OSAP with ESR. Liu and colleagues (2009) identified 19 genes (e.g. COL4A1, TFCP2, FHL3, SKIL, F2RL, PPP1R12B, LTBR, GADD45A, ACYP1) that enabled prediction of future disease activity (Liu et al., 2009).

3.2 Genes and signatures involved in early vs. late RA

Studies on differences in the PBMC gene expression profiles between early (disease duration less than 2 years) and established RA (with an average disease duration of 10 years) marked 53 genes with a three-fold difference in expression. A total of 9 genes, including colony stimulating factor 3 receptor, cleavage stimulation factor, and TGFβ receptor II, were upregulated in the early RA group. The deregulated genes were involved in immunity and cell cycle regulation. Since a quarter of the early arthritis genes overlapped with an influenza-induced gene set it was suggested that the early arthritis signature may partly reflect the response to an unknown infectious agent (Olsen et al., 2004). Accordingly, van der Pouw Kraan and colleagues observed similarity with a common virus response signature in a subset of RA patients (van der Pouw Kraan et al., 2008). Not surprisingly, the majority of these patients had an activated IFN-response program. Although no formal proof for a direct involvement of an infectious agent in RA, these findings suggest at least the presence of an activated pathogen response program in a subset of the patients. A comparative analysis between synovial tissues of RA patients suggested molecular differences between early (< 9 months) and late (> 4years) RA (Lequerre et al., 2009). However, due to the very limited sample size and role of confounding factors (age,

treatment, serology) the authors could not exclude a contribution of these factors for the observed differences.

Tsubaki and colleagues applied laser capture microscopy technology to evaluate the synovial lining cells in early (<12 months) and late RA (>5 years) patients (Tsubaki et al., 2005). First, they demonstrated that tissue heterogeneity within RA can already be observed in the phase of RA. The early RA patients could be divided in at least two different groups based on their gene expression profiles. A subgroup with exclusively early RA synovia was characterized by abundant expression of fibronectin1, B2-microglobulin, syndecan, cathepsin B, STAT-1, integrin-b2 and IFNGR2. The other group with cases of both long standing RA and early RA had an increased expression of CASP9, p53-induced gene 11, cathepsin G, CSF2RB, TNFRSF1A and IL-10RB. where expressed at a lower level. Gene expression profiling of synovial tissue of early versus long-standing rheumatoid arthritis suggest stage specific molecular patterns indicative for involvement of different pathophysiological mechanisms during the disease course of RA. A picture emerges that early RA is characterized by elevated expression of genes involved in immune-defense mechanisms, stress response and apoptosis, whereas long-standing RA showed increased expression of genes involved in proliferative processes.

3.3 Pharmacogenomics in RA towards personalized medicine

Gene expression profiling may also proof valuable for the predicting responses to therapy. In general a substantial percentage of patients do not respond to anti-rheumatic therapies, either DMARDs or biological. In particular expensive therapies with biologicals to target proinflammatory mediators of TNF, T- and B-lymphocytes, which are approved worldwide for the treatment of RA urged the need for predictors for therapy response. Clinical experience showed that the targeted therapies with biologicals are not effective for approximately 30-40% of the patients. Given the destructive nature of RA, the risk of adverse effects, and considerable costs for biologics therapy, there is a strong need to make predictions on success before the start of therapy.

In the late 1990s the term *pharmacogenomics* was introduced to frame gene expression profiling studies to delineate processes and identify biomarkers that correlate with the differential clinical outcome of pharmacological intervention. *Pharmacogenomics* is defined as: "The investigation of variations of DNA (genetics) and RNA (transcriptomics) characteristics as related to drug response". The value of pharmacogenomics in guiding clinical management has been highly appreciated in the field of oncology, as can be exemplified by the use of a gene signature to predict the response outcome of patients with breast cancer (Van 't Veer et al., 2002).

3.3.1 Pharmacogenomics of TNF-blockade

In the field of rheumatology specifically the response to TNF-blockers gained much attention. Currently 5 different TNF blockers are registered for clinical use in RA: A soluble TNF-receptor-Fc fusion protein Enbrel® (Etanercept), and 4 monoclonal antibodies directed against TNF Remicade® (Infliximab), Humira® (Adalumimab), Cinzia® (Certolizumab) and Simponi® (Golimumab).

3.3.2 Pharmacodynamics of TNF-blockade

Initial studies focused on the pharmacological effects of TNF blockade in the peripheral blood compartment in order to gain a comprehensive understanding of the mode of action. Global qualitative and quantitative pharmacogenomic analysis on peripheral blood cells suggest that all RA patients treated revealed an overall similar pharmacological response pattern, indicative of the presence of bioactive TNF in the circulation irrespective of clinical response (Van Baarsen et al., 2010c; Batliwalla et al., 2008). These findings suggest a model for the parallel presence of TNF-dependent and TNF-independent disease pathways in the individual patient, whereby the effect of anti-TNF therapy may be dependent on the relative contribution of the TNF-independent pathways. Meugnier and colleagues studied changes in PBMC gene expression after 12 wk of treatment with either etanercept or adalimumab from responder RA patients (Meugnier et al., 2011). Two hundred fifty-one genes displayed significant changes. Genes encoding S100A12 and A8, CD14 antigen, Selectin P, or ribosomal protein L39, reported to be upregulated in RA patients, were found to be decreased upon TNF-blockade. Pathway level analysis revealed that inflammation, immune response, apoptosis, protein synthesis, and mitochondrial oxido-reduction were the most affected pathways in response to anti-TNF-α treatment. Detailed analyses in search of (subtle) differences in the pharmacodynamic changes between responders and non-responders identified IFN-response genes as an informative sets of genes. The regulation of IFN-response genes by infliximab in RA turned out not to be as consistent as previously described for patients with SOJIA (Palucka et al., 2005), but varies between patients. A decrease in IFN-activity appears to be associated with good clinical responses (van Baarsen et al., 2010d; Sekiguchi et al., 2008). Koczan and colleagues reported that early downregulation of genes involved in different pathways and cellular processes such as TNFα signalling via NFκB, NFκB-independent signalling via cAMP, and the regulation of cellular and oxidative stress response (e.g. NFKBIA, CCL4, IL8, IL1B, TNFAIP3, PDE4B, PPP1R15A and ADM) were associated with a good clinical outcome to etanercept-based on ΔDAS >1.2. (Koczan et al., 2008). These studies demonstrated that observing the dynamics of the TNF-blocker intervention may provide insight into the biology of TNF blockade.

3.3.3 Prediction of response to TNF blockade

In an attempt to predict the response prior to treatment several studies have been performed. Initial analyses of synovial tissue gene expression profiles prior to the start of infliximab therapy suggested that patients with features of an activated immune status in their tissue compartment are more likely to benefit from anti-TNF treatment based on ΔDAS28 response criteria (Van der Pouw Kraan et al., 2008; Lindberg et al., 2006). Similar findings were reported for baseline serum markers associated with responsiveness (Hueber et al., 2009). In contrast, Badot and colleagues identified 439 genes involved in cell division and regulation of immune response (e.g. cytokines, chemokines and their receptors) that were associated with poor response based on EULAR criteria (Badot et al., 2009). A recent study performed in 62 RA patients by Lindberg et al. demonstrated an overrepresentation of lymphoid aggregates in EULAR responders (infliximab), in line with the earlier described relationship between inflammation and clinical outcome (Lindberg et al. 2010). These authors also caution for the confounding effect of cellular

complexity, suggesting the use of microdissected cell population for explorative genomic synovial tissue analyses.

In the ideal situation a prediction should be made prior to the start of therapy in an easily accessible biosample, such as peripheral blood. Ultimately, this may lead to apply therapy to each patient that is best suited for the patient, also called "personalized medicine". A number of studies focussed on the delineation of baseline differences in peripheral blood cells (whole blood and PBMC) with the purpose of predicting response (Lequerre et al.2006; Koczan et al., 2008; Sekiguchi et al. 2008; Tanino et al. 2009; Julia et al., 2009a; Bienkoska et al., 2009; Van Baarsen et al., 2010c, 2010d; Stuhlmuller et al., 2010). The results of these studies made clear that identification of predictors in the blood compartment is not trivial. Whereas several groups reported the absence of significant gene expression differences between responders and non-responders others were able to identify such markers. In 2006 Lequerre and colleagues report promising results of a study with 33 patients (13 patients in the test group and 20 patients in a validation group) using a in-house made microarray covering 10,000 unique genes, wherein they identified a gene set (consisting of AKAP9, COX7AL2, ELMOD2, EPS15, FBOX5, HLA-DPB1, LAMR1, MCP, MRLP22, MTCBP1, PFKFB4, PSMB9, PTPN12, QIL1, RASGRP3, RPL35, RSP16, RSP28, SCAM1 and TBL2) that predicts the response to infliximab based on the ΔDAS28>1.2 score after 3 months (Lequerre te al. 2006). A selected set of 8 gene transcripts (MTCBP1, AKAP9, RASGRP3, PTPN12, RSP28, HLA-DPB1, MRPL22 and EPS15) allowed them to predict the response with a sensitivity of 80% and a specificity of 100%. Julia and colleagues performed a gene expression analysis using a 47k gene bead array on whole blood RNA samples from 43 RA patients starting infliximab therapy (training set 29 and validation set 14) (Julia et al., 2009a). The clinical response was determined at week 14 using the EULAR criteria. They found an 8 gene classifier consisting of HLA-DRB3, SH2D1B, GNLY, CAMP, SLC2A3, IL2RB, MXD4 and TLR5 that predicted response with a sensitivity of 91.6% and specificity of 50%. In parallel, they observed a significantly higher number of CD4+CD25+ cells (i.e. regulatory T cells) in the responder group compared to the non responder group at baseline. Tanino and colleagues studied whole blood of 68 patients (training set 42 and validation set 26) using a 44k gene microarrays to study. They measured EULAR response criteria at week 14 and discovered a 10 transcript biomarker set consisting of PSPH, CLGN, C21orf58, TBC1D8, LOC643981, ATP51, ANKRD55, TMEM141 and an EST (A_32_P1144), that had a positive predictive value of 80% and a negative predictive value of 44.4% (Tinino et al., 2009). Bienkowska and colleagues constructed a predictor based on 8 gene (CLTB, MXRA7, CXorf52, COL4A3BP, YIPF6, BOD1L, SFRS2 and PGK1) using PBMC of 46 RA patients prior to the start of adalimumab, etanercept, or infliximab. Response status at 14 weeks was based on EULAR criteria (Bienkowska et al. 2009). The accuracy (86%), sensitivity and specificity of the predictor is confirmed by an independent validation data set of 11 patients. Stuhlmuller and colleagues identified a predictor for adalimumab monotherapy using purified monocytes from 77 RA patients (training set of 7 patients and validation set of 70 patients). Clinical outcome was based on the ACR response >20 criteria. They identified CD11c as predictive marker for adalumimab monotherapy (sensitivity 100% and specificity 91.7%). However, CD11c was not predictive of response to adalumimab in combination with MTX (Stuhlmuller et al. 2010).

An important conclusion is that the study results are inconsistent and that the predictive genes from the different studies showed no overlap. How relevant this is for proper prediction and whether this is the consequence of differences in cell source, technological variation (e.g. array platform), data analysis system used, differences in study populations and/or differences in clinical management of the patients remains to be determined. Clearly there is a high need for independent validation and standardization in protocols and technology to understand the basis for the varying results.

3.4 Pharmacogenomics of anakinra

Anakinra is an IL-1-receptor that neutralizes IL-1 activity. Anakinra has proven effective in reducing joint inflammation, pain and bone destruction. However, as with all therapies in RA a substantial percentage of patients do not respond to anakinra. A gene expression study of PBMC of 32 patients (training set of 14 patients and validation set of 18 patients) using an in-house made microarray with a complexity of 10,000 genes identified a 7 gene set (GTF2F2, CCT3, CROT, HNRPA3, ARL15, TMED5, NRG3) that proofed capable to identify responders and non-responders with a sensitivity of 80% and a specificity of 87.5% based on the ΔDAS28>1.2 criteria (Bansard et al., 2011). Strikingly, there was no overlap between genes that were differentially expressed between anakinra and infliximab specific responders and non-responders. (Lequerre et al. 2006) that would be expected based on the crossregulation of IL-1 and TNF. (Lequerre et al. 2006) These results are promising but require validation in a larger independent cohort.

3.5 Pharmacogenomics of rituximab

B cell depletion therapy via rituximab (an anti-CD20 antibody) was shown to be highly effective for suppression of disease activity in RA (Edwards et al., 2004). CD20 is expressed on immature to mature B cells, as well as memory cells, but not on stem cells or precursor cells and, importantly, antibody producing plasma cells. Currently, rituximab is generally used after failure of at least one TNF antagonist. Clinical studies have demonstrated that not all patients show a favorable response to rituximab therapy. Especially the fact that rituximab directly depletes specific B cell populations in all patients treated, irrespective of clinical outcome, has raised questions regarding the mechanism of action.

3.5.1 Pharmacodynamics of rituximab

Gene expression profiling on whole blood cells of 13 RA patients demonstrated that pharmacological responses under the influence of rituximab treatment are highly heterogeneous between patients (Vosslamber et al. 2011). A difference in the kinetics of only a cluster of type I IFN-response genes during rituximab treatment that distinguishes responders from non-responders was observed. Responders exhibited an increase in IFN-response activity after three months treatment with rituximab, whereas the IFN-response activity remained stable during treatment in the non-responders. Thus, whereas rituximab depletes B cells in all patients treated irrespective of their clinical response, gene expression data show that a drug-induced increase of type I IFN-response activity is associated with clinical response. Gutierrez-Roelens and colleagues showed that immunoglobulin genes and

genes involved in chemotaxis, leukocyte activation, and immune responses were dowregulated in the synovium at 3 months after the start of therapy (Gutierrez et al., 2011).

3.5.2 Prediction of response to rituximab

Gene expression studies were also performed to search for molecular biomarkers present in RA patients before the start of treatment in relation to the clinical outcome. Julia and colleagues studied whole blood, T cell and B cell profiles of 9 RA patients (Julia et al., 2009). The clinical outcome was determined using the relative improvement of DAS28 activity (relDAS28) after 24 weeks. Several genes were identified that were differential expressed between responders and non-responders at baseline (eg. ARG1, TRAF-1 and TLR4). In another study it was observed that the clinical outcome (based on ΔDAS28 and EULAR criteria) to rituximab is significantly associated with the IFN-response activity prior to the start of treatment (Vosslamber et al., 2011). Good responders have a low or absent IFN-response activity at baseline, whereas non-responders have an activated type I IFN-systems before the start of treatment. During therapy the IFN-activity increases in the IFN^{low} patients and remains stable in the IFN^{high} patients. The association between baseline type I IFN levels and clinical response is in line with previous findings wherein it was demonstrated in two different cohorts (n=20 and n=31) that patients with a low IFN signature had a significantly greater reduction in the DAS28 and more often achieved a EULAR response at weeks 12 and 24 (Thurlings et al., 2010). The clinical utility of the IFN-signature to predict non-responders was demonstrated in an independent study by Receiver Operating Characteristic (ROC)-curve characteristics analyses (unpublished observation).

Overall the above studies demonstrate that it may be feasible to select gene-expression based prognostic biomarkers for rituximab response outcome from the peripheral blood that have clinical relevance.

4. Gene expression in the preclinical (asymptomatic) phase of RA

One of the main goals in the prevention of the disease lies in early diagnosis followed by timely start of effective treatment in order to induce remission. Ideally, early diagnosis in the asymptomatic/preclinical phase is required. Several studies have documented the appearance of anti–citrullinated protein antibodies (ACPAs) and rheumatoid factor (RF) prior to the onset of RA (Rantapaa-Dahlqvist et al., 2003; Nielen et al., 2004)

The results of those studies indicate that ACPA and/or RF may serve as predictive biomarkers for the development for RA, which would allow the selection of candidates for preventive therapy. However, because RA does not ultimately develop in all ACPA and/or RF-positive individuals, the requirements to drive this process are likely to be different between individuals who are at risk (Klareskog et al., 2004). Hence, either additional factors are needed to result in a chronic inflammatory response ultimately leading to RA or some individuals may have a protective immune profile which suppresses disease development despite the presence of autoantibodies. The pathogenic or protective immune response might be selectively induced in susceptible individuals (Klareskog et al., 2004). It was suggested that increased levels of pro-inflammatory cytokines and/or chemokines are associated with the generation of ACPA as amplifiers of inflammatory responses (Rantapaa-

Dahlqvist, et al., 2007). The chemotactic activity could be related to cell migration. However, the exact nature of the pathogenic and/or protective response remains to be determined.

4.1 Gene expression profiles predict arthritis development, independent of ACPA levels

Gene expression profiling blood samples of persons at risk who do and who do not develop RA, revealed new insights in the pathogenic and protective mechanisms in the pre-clinical phase of RA. Gene expression signatures playing either a pathogenic or a protective role were identified in whole blood transcriptome of ACPA/RF positive patients at risk for RA. A total of 109 ACPA/RF positive arthralgia patients at risk for RA were clinically followed for progression to arthritis up to 5 years following inclusion, and clinical features were compared to whole genome expression profiles (Van Baarsen et al. 2010a).

Initial comparison of whole genome expression profiles of 19 autoantibody positive arthralgia patients at inclusion and healthy subject already revealed a marked increase in several immune-related processes in the arthralgia patients. A total of 554 genes were identified whose transcript levels deviated more than two-fold from the median expression level in at least four patients. Two-way hierarchical cluster analysis on these 554 differentially expressed genes clearly indicated that the autoantibody positive arthralgia patients were separated in different subgroups. Pathway-level analysis revealed that these gene clusters represent genes involved in different modes of immune activation e.g. IFN-response activity, B- cell mediated immunity, and chemokine and cytokine mediated signaling. These results were validated in an independent cohort of 90 arthralgia patients again showing considerable heterogeneity among the at risk individuals.

Interim follow-up analysis revealed that 20 autoantibody positive arthralgia patients out of the 109 patients followed had developed arthritis after a median of 7 months (IQR 4-15; median follow-up of all patients is 30 [IQR 22-39] months) in a median of 3 joints (IQR 3-5). Analyzing the distribution of arthritis converters (follow-up time of 12 months (n=102) and corrected for ACPA levels) over the different subgroups revealed that the subgroup that is characterized by an increased expression of genes involved in IFN-mediated immunity and cytokine-chemokine mediated immunity, is associated with arthritis development (OR 21.0; 95% C.I. 2.8-156.1; P=0.003) while the subgroup that is characterized by a relative increased expression of genes involved in B-cell mediated immunity is associated with absence of arthritis (OR 0.38; 95% C.I. 0.21-0.70; P=0.002).

It is speculated that the IFN response programme could be associated with activation of immature monocyte-derived DCs, which regulate deletion of autoreactive lymphocytes. Subsequently, IFN-matured DCs may activate autoreactive T cells, leading to autoreactive B-cell development, representing the first level of autoimmunity. Loss of tolerance may lead to autoantibody production. The decreased B cell gene expression with concomitant increased chemokine activity in the blood of at risk patients who converted to RA may be a consequence of extravasation of lymphocytes from the blood to sites of inflammation and/or lymphoid organs.

Collectively, these analyses reveal that autoantibody positive arthralgia patients with high expression of genes involved in IFN-mediated immunity or chemokine/cytokine mediated

immunity combined with a decreased expression of B-cell markers are more likely to develop arthritis.

5. Gene expression and genetics in RA

Sugino and colleagues studied the correlation between the RA susceptibility genes from genome-wide association studies (GWAS) - namely, CD244, PADI4, SLC22A2, PTPN22, CTLA4, TRF1/C5, CD40, CCL21 and STAT4 (Sugino et al., 2010). Gene expression analysis gene expression analysis in RA patients and healthy individuals from a Asian (Japanese) cohort showed that the expressions of four of these genes (CD244, PADI4, SLC22A2, and PTPN22) were significantly higher in RA patients than in healthy individuals, whereas *STAT4* expression was significantly downregulated in the RA group. Data on the upregulated genes is in agreement with results from i*n vitro* studies, which revealed the individual upregulation of CD244, PADI4, SLC22A2, and PTPN22 by the mutant alleles.

6. Systems biology

Unique to transcriptome analyses is the identification of gene signatures that represent biological networks that are relevant in disease pathogenesis and thus provide a starting point for a systems biology approach, i.e. a computational modeling approach aimed to understand the structure and dynamics of cellular and organismal functions. Successive research activities on these networks, together with approaches using complementary platforms such as (epi)genetics, multiplex fluorescence-activated cell sorting and advanced metabolomics/proteomics, will provide a complete insight into the mechanism and other network components of processes and pathways relevant to disease. Thus, besides identifying clinically relevant transcriptome markers, DNA-microarray technology provides a basis for an evidence-based systems biology approach to delineate pathogenic processes and reveal other relevant markers. Meta-analysis methods will be instrumental in helping to select those exploratory markers for further biomarker validation, which will pave the way for clinical development and benefit patients. Wu and colleagues devised a method to construct a systemic network of interactions of the processes ongoing in patients affected by RA (Wu et al., 2010) . The network is based on high-throughput data from gene expression profiling and other technology platforms, refined semi-automatically with carefully curated literature-based information. This global network has then been topologically analysed, as a whole and tissue-specifically, in order to translate the experimental molecular connections into topological motifs meaningful in the identification of tissue-specific markers and targets in the diagnosis, and possibly in the therapy, of RA. They demonstrated some nodes in the network that prove to be topologically important, in particular AKT2, IL6, MAPK1 and TP53. Moreover they suggest CRKL as a novel potentially relevant molecule for the diagnosis or treatment of RA. This type of finding proves that the massive amounts of data from high-throughput technologies like gene expression sources create an excellent basis for in silico analyses able to produce highly refined hypotheses, based on vast experimental data, to be tested further and more efficiently. The network is freely available in a standardised and easily exportable .xml Cell Designer format at 'www.picb.ac.cn/ClinicalGenomicNTW/temp.html' and 'www.celldesigner.org'

Moreover, Xing and colleagues described a data analysis strategy for predicting gene expression measures using a combination of comprehensive genotyping, whole blood gene expression profiles and the component of clinical measures (Xing et al., 2011). They identified a total of 22 genes that may have a role in modulating DAS28 score; 6 genes (including CD86, a T-cell costimulatory molecule) could be an alternative target to TNF-blockers and 59 genes are predicted to affect tender and swollen joints. The genes and pathways identified in the networks ensembles represent are potentially promising targets for future investigations.

7. Concluding remarks

Gene expression profiling approaches have fuelled insight into the complexity of RA pathogenesis and provide a framework to identify pathogenic processes and biomarkers as promising tool for future clinical applications. Molecular profiling of blood cells and tissue samples of RA patients has already revealed important information, such as e.g. an activated IFN-system in a subset of patients, that is likely to contribute to the spectrum of diversity in RA. Future efforts are directed on integration of data from different technology platforms combined with information from literature towards a systems biology approach to construct a systemic network of interactions between molecular interactions that reflect disease processes that are ongoing in RA.

8. References

Aidinis V, Carninci P, Armaka M, et al., Cytoskeletal rearrangements in synovial fibroblasts as a novel pathophysiological determinant of modeled rheumatoid arthritis. PLoS Genet 2005, 1:e48.

Badot V, Galant C, Nzeusseu Toukap A, et al., Gene expression profiling in the synovium identifies a predictive signature of absence of response to adalimumab therapy in rheumatoid arthritis. Arthritis Res Ther. 2009;11(2):R57.

Bansard C, Lequerre T, Derambure C, et al., Gene profiling predicts rheumatoid arthritis responsiveness to IL-1Ra (anakinra). Rheumatology 2011;50:283-292

Batliwalla FM, Baechler EC, Xiao X, et al., Peripheral blood gene expression profiling in rheumatoid arthritis. Genes Immun 2005, 6:388-397.

Batliwalla F, Li W, Bienkowska J, Damle A, Khalili H, Hueber W, Allaire M, Mcrann M, Robinson W, Kern M, Carulli JP, Gregersen PK: Differential peripheral blood gene expression profile of rheumatoid arthritis in response to anti-TNF treatment [abstract]. Arthritis Rheum 2007, 56:S700

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 USA 2003, 100:2610-2615

Bienkowska JR, Dalgin GS, Batliwalla F, et al., Convergent Random Forest predictor: methodology for predicting drug response from genome-scale data applied to anti-TNF response. Genomics. 2009 Dec;94(6):423-32.

Bos CL, van Baarsen LGM, Timmer TCG, et al., Molecular subtypes of systemic sclerosis in association with anti-centromer antibodies and digital ulcers. Genes Immun. 2009 Apr;10(3):210-8

Bovin LF, Rieneck K, Workman C, Nielsen H, Sorensen SF, Skjodt H, Florescu A, Brunak S, Bendtzen K: Blood cell gene expression profiling in rheumatoid arthritis. Discriminative genes and effect of rheumatoid factor. Immunol Lett 2004, 93:217-226.

Brazma A, Hingamp P, Quackenbush J, et al., Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet. 2001; 29(4):365-371

Brouty-Bové D, Pottin-Clemenceau C, Doucet C et al., Chemokines and CD40 expression in human fibroblasts. Eur. J. Immunol. 2000;30(3):914-919

Lu C, Xiao C, Chen G, et al., Cold and heat pattern of rheumatoid arthritis in traditional Chinese medicine: distinct molecular signatures indentified by microarray expression profiles in CD4-positive T cell. Rheumatol Int. 2010, [E-pub ahead]

Devauchelle V, Marion S, Cagnard N, et al., DNA microarray allows molecular profiling of rheumatoid arthritis and identification of pathophysiological targets. Genes Immun 2004, 5:597-608.

Edwards JC, Szczepanski L, Szechinski J, et al., Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med. 2004, 350:2572-2581.

Firestein GS and Zvaifler NJ. How important are T cells in chronic rheumatoid synovitis?: II. T cell-independent mechanisms from beginning to end. Arthritis Rheum. 2002 Feb;46(2):298-308.

Gallagher J, Howlin J, McCarthy C, et al., Identification of Naf1/ABIN-1 among TNF-alpha-induced expressed genes in human synoviocytes using oligonucleotide microarrays. FEBS Lett. 2003 Sep 11;551(1-3):8-12.

Gallagher J, Howlin J, McCarthy C, et al., Identification of Naf1/ABIN-1 among TNF-alpha-induced expressed genes in human synoviocytes using oligonucleotide microarrays. FEBS Lett 2003, 551:8-12.

Galligan CL, Baig E, Bykerk V, et al., Distinctive gene expression signatures in rheumatoid arthritis synovial tissue fibroblast cells: correlates with disease activity. Genes Immun. 2007 Sep;8(6):480-491

Guttierrez-Roelens I, Galant C, Theate I, et al., Rituximab treatment induces the expression of genes involved in healing processes in the rheumatoid arthritis synovium. Arthritis Rheum. 2011 May;63(5):1246-1254.

Heller RA, Schena M, Chai A, et al. Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. Proceedings of the National Academy of Sciences of the United States of America 1997; 94: 2150–2155

Higgs BW, Liu Z, White B, et al., Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis. 2011 Jul 28. [Epub ahead of print]

Hirohata S, Yanagida T, Nampei A et al., Enhanced generation of endothelial cells from CD34+ cells of the bone marrow in rheumatoid arthritis: possible role in synovial neovascularization. Arthritis Rheum 2004;50, 3888-3896

Hogaboam CM, Steinhauser ML, Chensue SW et al., Novel roles for chemokines and fibroblasts in interstitial fibrosis. Kidney Int. 1998 54(6):2152-2159

Hueber W, Tomooka BH, Batliwalla F, et al., Blood autoantibody and cytokine profiles predict response to anti-TNF therapy in rheumatoid arthritis. Arthritis Res Ther 2009, 11:R76.

Huber R, Hummert C, Gausmann U, et al., Identification of intra-group, inter-individual, and gene-specific variances in mRNA expression profiles in the rheumatoid arthritis synovial membrane. Arthritis Res Ther. 2008;10(4):R98

Jiang M, Xiao C, Chen G, et al., Correlation between cold and hot pattern in traditional Chinese medicine and gene expression profiles in rheumatoid arthritis. Front Med. 2011 ,5;:219-228.

Jongen-Lavrencic M, Peeters HR, Wognum A, Vreugdenhil G, Breedveld FC, Swaak AJ. Elevated levels of inflammatory cytokines in bone marrow of patients with rheumatoid arthritis and anemia of chronic disease. J Rheumatol. 1997;24, 241-245

Julià A, Barceló M, Erra A, Palacio C, Marsal S. Identification of candidate genes for rituximab response in rheumatoid arthritis patients by microarray expression profiling in blood cells. Pharmacogenomics 2009a, 10.1697-708

Julià A, Erra A, Palacio C, et al., An eight-gene blood expression profile predicts the response to infliximab in rheumatoid arthritis. PLoS One. 2009b Oct 22;4(10):e7556.

Kasperkovitz PV, Timmer T, Verbeet NL, et al., Fibroblast-like synoviocytes show the imprint of synovial tissue heterogeneity in rheumatoid arthritis: Evidence for a link between the presence of myofibroblast-like cells and high inflammatory synovitis. Arthr Rheum. (2005) 52:430-441

Kasperkovitz PV, Verbeet NL, Smeets TJ, et al., Activation of the STAT1 pathway in rheumatoid arthritis. Ann Rheum Dis. 2004; 63(3):233-239

Kato H, Matsumine A, Wakabayashi T, et al., Large-scale expression profiles, differentially repressed in osteoarthritis synovium of the knee joint using cDNA microarray technology. Biomarkers 2007;12:384-402

Klareskog L, Alfredsson L, Rantapaa-Dahlqvist S, et al., What precedes development of rheumatoid arthritis? Ann Rheum Dis 2004; 63 Suppl 2: ii28–31.

Koczan D, Drynda S, Hecker M, et al., Molecular discrimination of responders and nonresponders to anti-TNF alpha therapy in rheumatoid arthritis by etanercept. Arthritis Res Ther. 2008;10(3):R50.

Lee HM, Sugino H, Aoki C, et al., Abnormal networks of immune response-related molecules in bone marrow cells from patients with rheumatoid arthritis as revealed by DNA microarray analysis. Arthritis res Ther. 2011 Jun 16;13(3):R89

Lequerré T, Gauthier-Jauneau AC, Bansard C, et al., Gene profiling in white blood cells predicts infliximab responsiveness in rheumatoid arthritis. Arthritis Res Ther. 2006;8(4):R105

Lequerré T, Bansard C, Vittecoq O, et al., Early and long-standing rheumatoid arthritis: distinct molecular signatures identified by gene-expression profiling in synovia. Arthritis Res Ther. 2009;11(3):R99

Liao H, Wu J, Kuhn E, et al., Use of mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of patients with rheumatoid arthritis. Arthritis Rheum. 2004, 50: 3792–3803

Lindberg J, af Klint E, Ulfgren AK, et al., Variability in synovial inflammation in rheumatoid arthritis investigated by microarray technology. Arthritis Res Ther 2006a, 8:R47

Lindberg J, af Klint E, Catrina AI, et al., Effect of infliximab on mRNA expression profiles in synovial tissue of rheumatoid arthritis patients. Arthritis Res Ther 2006b, 8:R179.

Lindberg J, Wijbrandts CA, van Baarsen LG, et al., The gene expression profile in the synovium as a predictor of the clinical response to infliximab treatment in rheumatoid arthritis. PLoS One. 2010 Jun 25;5(6):e11310.

Liu Z, Sokka T, Maas K, et al., Prediction of Disease Severity in Patients with early rheumatoid arthritis by gene expression profiling. Human Genomics and Proteomics 2009,484351

Maas K, Chen H, Shyr Y, et al., Shared gene expression profiles in individuals with autoimmune disease and unaffected first-degree relatives of individuals with autoimmune disease. Hum Mol Genet 2005, 14:1305-1314.

Mavragani CP, La DT, Stohl W, et al., Association of the response to tumor necrosis factor antagonists with plasma type I interferon activity and interferon-beta/alpha ratios

in rheumatoid arthritis patients: a post hoc analysis of a predominantly Hispanic cohort. Arthritis Rheum. 2010 Feb;62(2):392-401

Mavragani CP, Crow MK. Activation of type I interferon pathway in primary Sjogren's syndrome. J Autoimmun 2010; 35:225-231.

Mesko B, Poliska S, Szegedi A, et al., Peripheral blood gene expression patterns discriminate among chronic inflammatory diseases and healthy controls and identify novel targets. BMC Med Genomics. 2010 May 5;3:15

Meugnier E, Coury F, Tebib J, et al., Gene expression profiling in peripheral blood cells of patients with rheumatoid arthritis in response to anti-TNF-alpha treatments. Physiol Genomics. 2011 Apr 12;43(7):365-371

Nakamura N, Shimaoka Y, Tougan T, et al., Isolation and expression profiling of genes upregulated in bone marrow-derived mononuclear cells of rheumatoid arthritis patients. DNA Research. 2006;13, 169-183

Neumann E, Riepl B, Knedla A, et al., Cell culture and passaging alters gene expression pattern and proliferation rate in rheumatoid arthritis synovial fibroblasts. Arthritis Res Ther. 2010;12(3):R83.

Nielen MM, van Schaardenburg D, Reesink HW et al., Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthr Rheum 2004; 50, 380-386

Nzeusseu TA, Galant C, Theate I, et al., Identification of distinct gene expression profiles in the synovium of patients with systemic lupus erythematosus. Arthritis Rheum 2007, 56:1579-1588.

Ochi T, Hakomori S, Adachi M et al. The presence of a myeloid cell population showing strong reactivity with monoclonal antibody directed to difucosyl type 2 chain in epiphyseal bone marrow adjacent to joints affected with rheumatoid arthritis (RA) and its absence in the corresponding normal and non-RA bone marrow. J Rheumatology 1998;15, 1609-1615

O'Hanlon TP, Rider LG, Gan L, et al., Gene expression profiles from discordant monozygotic twins suggest that molecular pathways are shared among multiple systemic autoimmune diseases. Arthritis Res Ther. 2011 Apr 26;13(2):R69.

Olsen N, Sokka T, Seehorn CL, et al., A gene expression signature for recent onset rheumatoid arthritis in peripheral blood mononuclear cells. Ann Rheum Dis. 2004,63;1387-1392

Palucka AK, Blanck JP, Bennet L, et al., Cross-regulation of TNF and IFN-alpha in autoimmune diseases. Proc. Natl. Acad. Sci USA 2005;102,3372-3377

Pohlers D, Beyer A, Koczan D, et al., Constitutive upregulation of the transforming growth factor-beta pathway in rheumatoid arthritis synovial fibroblasts. Arthritis Res Ther. 2007;9(3):R59.

Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthr Rheum 2003;48, 2741-2749

Rantapaa-Dahlqvist S, Boman K, Tarkowski A, Hallmans G. Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis. Ann Rheum Dis 2007; 66: 121–3.

Sekiguchi N, Kawauchi S, Furuya T, et al., Messenger ribonucleic acid expression profile in peripheral blood cells from RA patients following treatment with an anti-TNF-

alpha monoclonal antibody, infliximab. Rheumatology (Oxford). 2008 Jun;47(6):780-788.

Sha N, Vannucci M, Brown PJ, et al.,. Gene selection in arthritis classification with large-scale microarray expression profiles. Comp Funct Genomics. 2003;4(2):171-181.

Smith RS, Smith TJ, Blieden TM, et al., Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. Am. J. Pathol. 1997; 151(2):317-322

Szodoray P, Alex P, Frank MB, et al., A genome-scale assessment of peripheral blood B-cell molecular homeostasis in patients with rheumatoid arthritis. Rheumatology (Oxford). 2006 Dec;45(12):1466-1476.

Taberner M, Scott KF, Weininger L, et al., Overlapping gene expression profiles in rheumatoid fibroblast-like synoviocytes induced by the proinflammatory cytokines interleukin-1 beta and tumor necrosis factor. Inflamm Res. 2005 Jan;54(1):10-6.

Tan FK, Zhou X, Mayes MD, et al., Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. Rheumatology (Oxford) 2006;45:694–702

Stuhlmüller B, Häupl T, Hernandez MM, et al., CD11c as a transcriptional biomarker to predict response to anti-TNF monotherapy with adalimumab in patients with rheumatoid arthritis. Clin Pharmacol Ther. 2010; Mar;87(3):311-321

Sugino H, Lee HM, Nishimoto N. DNA microarray analysis of rheumatoid arthritis susceptibility genes identified by genome-wide association studies. Arthritis Res Ther. 2010;12(2):401. Epub 2010 Mar 12. Erratum in: Arthritis Res Ther. 2010;12(3):403.

Tanabe M, Ochi T, Tomita T, Suzuki R, Sakata T, Shimaoka Y, Nakagawa S, Ono K., Remarkable elevation of interleukin 6 and interleukin 8 levels in the bone marrow serum of patients with rheumatoid arthritis.J Rheumatolog 1994, 21, 830-835

Tanino M, Matoba R, Nakamura S, et al., Prediction of efficacy of anti-TNF biologic agent, infliximab, for rheumatoid arthritis patients using a comprehensive transcriptome analysis of white blood cells. Biochem Biophys Res Commun. 2009 Sep 18;387(2):261-5.

Takemura S, Braun A, Crowson C, et al. Lymphoid neogenesis in rheumatoid synovitis. J Immunol 2001;167:1072–1080.

Teixeira VH, Olaso R, Martin-Magniette ML, et al., Transcriptome analysis describing new immunity and defense genes in peripheral blood mononuclear cells of rheumatoid arthritis patients. PLoS One. 2009 Aug 27;4(8):e6803.

Thurlings RM, Boumans M, Tekstra J, et al., The relationship between the type I interferon signature and the response to rituximab in rheumatoid arthritis. Arthritis Rheum. 2010, 62:3607-3614

Timmer TC, Baltus B, Vondenhoff M, et al., Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected by genomics technology: identification of the interleukin-7 signaling pathway in tissues with lymphoid neogenesis. Arthritis Rheum 2007, 56:2492-2502.

Tsubaki T, Arita N, Kawakami T, Shiratsuchi T, et al., Characterization of histopathology and gene-expression profiles of synovitis in early rheumatoid arthritis using targeted biopsy specimens. Arthritis Res Ther. 2005;7(4):R825-36.

Turrel-Davin F, Tournadre A, Pachot A, et al., FoxO3a involved in neutrophil and T cell survival is overexpressed in rheumatoid blood and synovial tissue. Ann Rheum Dis. 2010 Apr;69(4):755-760

Ungethuem U, Haupl T, Witt H, et al., Molecular signatures and new candidates to target the pathogenesis of rheumatoid arthritis Physiol. Genomics 2010;42A:(4) 267-282

van Baarsen LG, van der Pouw Kraan TC, Kragt JJ, et al., A subtype of multiple sclerosis defined by an activated immune defense program. Genes Immun. 2006;7:522–531

van Baarsen LG, Bos WH, Rustenburg F, et al., Gene expression profiling in autoantibody-positive patients with arthralgia predicts development of arthritis. Arthritis Rheum. 2010a;62:694-704

van Baarsen LG, Wijbrandts CA, Timmer TC, et al. Synovial tissue heterogeneity in rheumatoid arthritis in relationship to disease activity and biomarkers in peripheral blood. Arthritis Rheum. 2010b ;62:1602-1607

Van Baarsen LG, Wijbrandts C, Gerlag D, et al., Pharmacogenomics of infliximab treatment using peripheral blood cells of patients with rheumatoid arthritis. Genes Immun.2010c;11:622-629

van Baarsen LG, Wijbrandts CA, Rustenburg F, et al., Regulation of IFN response gene activity during infliximab treatment in rheumatoid arthritis is associated with clinical response to treatment. Arthritis Res Ther. 2010d;12:R11

Van der Pouw Kraan TCTM, Van Galen FA, Huizinga TWJ, et al., Discovery of distinctive gene expression profiles in rheumatoid arthritis using cDNA microarray technology: Evidence for the existence of multiple pathways of tissue destruction and repair. Genes & Immunity 2003a;4, 187-196

Van der Pouw Kraan TCTM, Van Galen FA, Kasperkovitz PV, et al., Rheumatoid arthritis is a heterogeneous disease: Evidence for differences in the activation of the STAT1 pathway between rheumatoid tissues. Arthr. Rheum. 2003b;48, 2132-2145

Van der Pouw Kraan TC, Wijbrandts CA, Van Baarsen LG, et al., Rheumatoid Arthritis subtypes identified by genomic profiling of peripheral blood cells: Assignment of a type I interferon signature in a subpopulation of patients. Ann Rheum Dis 2007;66, 1008-1014

Van der Pouw Kraan TC, Van Baarsen LG, Wijbrandts CA, et al., Expression of a pathogen-response program in peripheral blood cells defines a subgroup of Rheumatoid Arthritis patients. Genes Immun. 2008;9:16-22

Van 't Veer LJ, Dai H, van de Vijver MJ, et al., Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-536

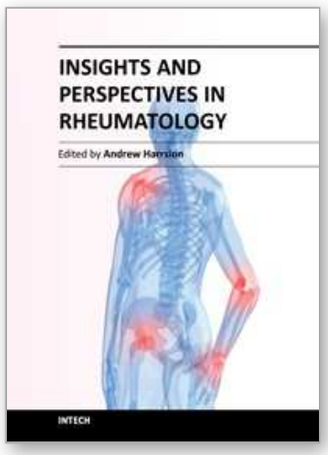
Vosslamber S, Raterman HG, van der Pouw Kraan CTM, Schreurs MWJ, von Blomberg BME, Nurmohamed MT, Lems WF, Dijkmans BAC, Voskuyl AE, Verweij CL. Pharmacological induction of IFN type I activity following therapy with rituximab determines clinical response in rheumatoid arthritis. Ann Rheum Dis 2011,70(6):1153-1159

Watanabe N, Ando K, Yoshida S, et al., Gene expression profile analysis of rheumatoid synovial fibroblast cultures revealing the overexpression of genes responsible for tumor-like growth of rheumatoid synovium. Biochem Biophys Res Commun 2002 Jun 28;294(5):1121-1129.

Wu G, Zhu L, Dent JE, Nardini C. A comprehensive molecular interaction map forrheumatoid arthritis. PLoS One. 2010 Apr 16;5(4):e10137. Erratum in: PLoS One.2010;5(4). doi:10.1371/annotation/f67a90fb-3e4e-4484-bffe-fcfafbfe88c7

Xing H, McDonagh PD, Bienkowska J, et al., Causal modeling using network ensemble simulations of genetic and gene expression data predicts genes involved in rheumatoid arthritis. PLoS Comput Biol. 2011 Mar;7(3):e1001105.

Zanders ED, Goulden MG, Kennedy TC, Kempsell KE: Analysis of immune system gene expression in small rheumatoid arthritis biopsies using a combination of subtractive hybridization and high-density cDNA arrays. J Immunol Methods 2000, 233:131-140.



Insights and Perspectives in Rheumatology

Edited by Dr. Andrew Harrison

ISBN 978-953-307-846-5
Hard cover, 274 pages
Publisher InTech
Published online 13, January, 2012
Published in print edition January, 2012

This book offers a range of perspectives on pathogenesis, clinical features and treatment of different rheumatic diseases, with a particular focus on some of the interesting aspects of Sjögren's syndrome. It contains detailed and thorough reviews by international experts, with a diverse range of academic backgrounds. It will also serve as a useful source of information for anyone with a passive interest in rheumatology, from the genetic and molecular level, through to the psychological impact of pain and disability.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Cornelis L. Verweij and Saskia Vosslamber (2012). Gene Expression Profiling in Rheumatoid Arthritis, Insights and Perspectives in Rheumatology, Dr. Andrew Harrison (Ed.), ISBN: 978-953-307-846-5, InTech, Available from: http://www.intechopen.com/books/insights-and-perspectives-in-rheumatology/gene-expression-profiling-in-rheumatoid-arthritis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen