Genomic Stratification by HLA-DRB4 Expression Identifies Innate and Adaptive Immune Predictors as Differential Predictors of Response to Methotrexate in Rheumatoid Arthritis – A Strategy to Detect Predictors of Methotrexate Response in Early Rheumatoid Arthritis

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BACKGROUND

Variability of clinical course and response to therapy in Rheumatoid Arthritis (RA) suggests differences in molecular mechanisms depending on stage and sub-entity of the disease. Most dominant molecular discriminators are HLA-DRB4 shared epitope (SE), and the status of rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) development, which are accompanied by autoantibodies. Identification of cellular & molecular processes (CMP) would be an option to minimize such effects, to determine the most promising therapy for each individual patient subgroup, and last but not least to reduce socio-economic costs.

OBJECTIVES

Early characterization of MTX treatment was investigated to exclude major therapeutic influence. By comparing whole blood transcriptomes between subsequent responders and non-responders we aimed to unravel molecular mechanisms with influence on MTX outcome of RA. This study aimed at defining CMPs, as well as to identify predictive mRNA and miRNA candidate genes in PAxGene whole blood samples of early RA patients for the response to future treatment with the anchor drug MTX.

METHODS

Whole blood PAxGene samples from 52 patients with early RA were obtained in the HITHARD and in the ArtroMark study. Exclusively, patients who had received no or only low doses of steroids were analyzed. Clinical characteristics assessed at baseline and after 16 weeks included RF, ACPA, DAS28 and EULAR responder status. In good responders (MR) and non-responders (NR), Total RNA was globally reduced and processed according to standard protocols for hybridization to Affymetrix HG U133 Plus 2.0 microarrays. Biosstatistical methods included MASS 5.0 and RMA algorithms with limma, lasso and Wilcoxon tests. For functional interpretation, expression of candidate genes was tested in various blood cell types and stimulation conditions using own and GEO reference transcriptomes. Marker selection was validated by qPCR with commercial primers and four different housekeeping genes (β-actin, γ-actin, GAPDH, HPBPD).

RESULTS

Assuming impact by immunological or genetic characteristics on pathomechanisms and treatment outcome, patients were grouped either by gender, RF, ACPA, SE or the haplotype-specific HLA transcripts DRB4 (203290_at) and DQA1 (209728_at). In each subgroup, responders and non-responders were compared and genes ranked by frequency of change and fold change. For each subgroup comparison, the best 100 genes increased and 100 decreased in responders were selected. Scoring differential expression of all 200 genes for each HLA-DRB4+ patient revealed a high correlation with clinical response. Moderate responders represented independent samples and were located between responders and non-responders (Fig. 1).

Validation of HLA-DRB4- candidate genes by “leave-one-out” excluded each patient at least once and frequently combined also with innate patterns generated 12 different comparisons based on MAS5.0/BioRetis algorithms (1). Each validation gene set improved pattern selection. In HLA-DRB4- patients, genes increased in GR were related to phagocytes and bone marrow activation, and overlapped with the complete group comparison set by 66-80% for increased and 67-82% for decreased in responders. Of the complete group comparison set by HLA-DRB4- patients, 96.5% (range: 91-100%) were ranked within the top 250 different subgroups defined by gender, status of RF, ACPA, SE, HLA-DRB4 or DQA1 identified molecular predictors of response. Insufficient discriminatory power. Generating 12 different subgroups including RF, ACPA, SE, HLA-DRB4- patients, all indicating sub-entities with influence on severity of disease and treatment outcome. Methotrexate (MTX) is one of the most prescribed long-term effective disease-modifying anti-rheumatic drugs (DMARDs) and therefore considered as the standard medication for the treatment of RA. Nonetheless, about 40-50% of the patients do not show adequate improvement under MTX therapy, while some patients even suffer from adverse and toxic side effects like pulmonary, hepatic, renal or bone marrow abnormalities. Identification of cellular & molecular processes (CMP) would be an option to minimize such effects, to determine the most promising therapy for each individual patient subgroup, and last but not least to reduce socio-economic costs.

CONCLUSION

An early outcome prediction of successful therapy, in particular with MTX as anchor drug in RA even after initiation of the disease, provides the opportunity for individually customized medications and may therefore reduce costs and prevent serious side effects of current RA treatment strategies. Identification of specific predictive response marker genes such as CD14 for anti-TNF monotherapy (2, 3), leads to a substantial interest to define predictive biomarkers for MTX monotherapy (4), as well. Furthermore, the combination of those markers is an approach to calculate the outcome of the standard combination therapy with both drugs. Combined genetic, genomic cellular stratification revealed transcriptional patterns that indicate different molecular pathomechanisms in RA depending on response to MTX therapy. Interestingly, the group responding to MTX was dominated by phagocytosis but not lymphocyte activity, which may indicate an important contribution of innate triggers to the pathomechanisms in RA.

REFERENCES

3. Patnaik and Tfn (20096809); EP1516714 (201691991); and Patent Nk (2016000038); EP152072208; European Patent 2015000038; EP152072208
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