Univerzita Karlova v Praze
1. lékařská fakulta

Autoreferát disertační práce

Molekulární aspekty muskuloskeletálních onemocnění a význam malých regulačních RNA

Molecular aspects of musculoskeletal diseases and the role of small regulatory RNAs

MUDr. Lenka Pleštilová

Praha, 2015

Doktorské studijní programy v biomedicíně

Univerzita Karlova v Praze a Akademie věd České republiky

Obor: Imunologie

Předseda oborové rady: Prof. RNDr. Vladimír Holáň, Dr.Sc.

Školicí pracoviště: Revmatologický ústav, Klinika revmatologie 1. LF UK, Na Slupi 4, 128 50, Praha 2

Školitel: Prof. MUDr. Vencovský Jiří, DrSc.

Konzultant: Dr. Astrid Jüngel, PD

Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

ABSTRAKT

Revmatologická onemocnění bývají chronická, bolestivá a do určité míry invalidizující. Porozumění mechanismům jejich patogeneze je zatím velmi neúplné. Hyper-reaktivní imunitní systém a porucha autotolerance jsou zřejmě doplněny lokálními faktory, které způsobí, že některé klouby/svaly bývají postižené více a jiné méně. Vše je výsledkem složité sítě interakcí mezi imunitními buňkami, kloubními fibroblasty, chondrocyty, osteocyty, myocyty a dalšími buňkami.
V předložené dizertační práci jsem se zaměřila na tři molekulární aspekty patogeneze revmatologických onemocnění: regulační RNA, S100 proteiny a autoprotilátky. V teoretické části jsem shrnula, co je známo o vzniku, funkci a významu těchto molekul v revmatologii. Ve výzkumné části předkládám šest originálních publikací a jedno review o roli těchto molekul při vývoji revmatoidní artritidy (RA) a idiopatické zánětlivé myopatie (IZM).

Mezi hlavní výsledky práce patří studie popisující expresi PIWI-interagujících RNA (piRNA) v synoviálních fibroblastech pacientů s RA. piRNA jsou malé regulační RNA, které v komplexu s PIWIL proteiny regulují genovou expresi a tlumí transpozómy. Exprese piRNA molekul byla dlouho považována za výhradní vlastnost zárodečných a nádorových buněk. My jsme detekovali 268 piRNA molekul v RA synoviálních fibroblastech (SF), a popsali jejich deregulaci v porovnání se SF od pacientů s osteoartrózou. Dále popisujeme přítomnost PIWIL4 proteinu v jádře synoviálních fibroblastů a jeho regulaci prozánětlivými cytokiny a TLR-ligandy. V dalších studiích jsme zkoumali možné využití cirkulujících mikroRNA jako biomarkerů u IZM a S100A8/9 jako biomarkeru u časné RA. Při výzkumu role S100A4 proteinu zvaného metastazin se nabízela souvislost s malignitou u pacientů s IZM (tzv. cancer associated myositis). Tuto jsme však nepotvrdili, popsali jsme ale překvapivou korelaci sérových hladin S100A4 s aktivitou základního onemocnění. Výsledky stanovení autoprotilátek metodou radioimunoprecipitace byly využity pro popisnou studii o autoprotilátkových asociacích a klinické podobě artritidy jako symptomu IZM a dále pro zprávu o stoupající incidenci imunitně podmíněné nekrotizující myopatie.

Výsledky této práce by měly přispět k lepšímu pochopení patogeneze muskuloskeletálních onemocnění a zlepšení jejich léčby.

Klíčová slova:
Revmatoidní artritida, myozitida, miRNA, piRNA, S100 proteiny, autoprotilátky

ABSTRACT

Rheumatic diseases are common, usually chronic, painful and to some extent invalidating medical conditions. Understanding of the disease pathogenesis is still very fragmentary. Hyperreactivity of the immune system and defect of autotolerance are probably contributed by local factors, which helps to explain, why some joints/muscles are more affected than others. All this results from a complex net of interactions between immune cells, synovial fibroblasts, chondrocytes, osteocytes, myocytes and other cells.

In the submitted PhD thesis I have focused on three groups of molecules: regulatory RNAs, S100 proteins and autoantibodies. In the theoretical part, I sum up the current knowledge on their biogenesis, function and the role in rheumatology. In the investigative part, I present six original publications and one review on the role of those molecules in development of rheumatoid arthritis (RA) and idiopathic inflammatory myositis (IIM).

One of the main studies was focused on expression of PIWI-interacting RNAs (piRNAs) in RA synovial fibroblasts (SF). piRNAs are small regulatory RNAs which in complex with PIWIL proteins regulate gene expression and silence transpozoms. piRNA expression was considered to be limited to germline and cancer cells. We have found 267 PIWI-interacting RNAs to be expressed in RA synovial fibroblasts and described their deregulation when compared to osteoarthritis SF. We have also described the presence of PIWIL4 protein in the nuclei of synovial fibroblasts and its regulation by proinflammatory cytokines and TLT-ligands. We have also studied a possible use of miRNAs as biomarkers in IIM and use of S100A8/9 as biomarker in early RA. When investigating the function of S100A4, a protein also known as metastasin, a possible link with malignity in cancer associated myositis was considered. We were not able to confirm this association, but somewhat surprisingly, we observed a correlation of the S100A4 serum levels with clinical activity in myositis. Autoantibody results detected by the radioimmunoprecipitation method were used for a descriptive study on autoantibodies associations and clinical form of arthritis in IIM and for a report on the increasing incidence of the immune mediated necrotizing myopathy.

Results of this PhD. thesis aim to improve our understanding of pathogenesis of musculosceletal diseases and their treatment.

Key words:
Rheumatoid arthritis, myositis, miRNA, piRNA, S100 proteins, autoantibodies

CONTENTS

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease causing joint destruction as well as systemic inflammation. Estimated prevalence of RA in developed countries is 0.5-1% [1]. Environmental factors such as smoking can lead in the genetically predisposed persons (polymorphisms in HLA-DRB1) to the development of a specific immunity against citrullinated peptides and to the production of autoantibodies [2]. Autoantibodies against citrullinated peptides have been shown to have a pathogenic role in RA [3]. Beside the immune cells, synovial fibroblasts (SF) are an important effector cells in RA. SF produce inflammatory cytokines, chemokines and matrix degrading enzymes which attract inflammatory cells and destroy the cartilage in RA joint [4, 5]. RASF show "activated" phenotype possibly due to epigenetic modifications [6, 7]. The current treatment strategy in RA is to decrease the inflammation as fast as possible to prevent joint deformities and cardiovascular complications. Nevertheless the mortality of RA patients is still about 1.5-times increased compared to healthy population [8]. Research on the molecules involved in the pathogenesis of RA will hopefully lead to the discovery of new therapy targets and/or biomarkers, which enable further individualisation of the treatment.

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of acquired chronic muscle disorders, with main subtypes dermatomyositis, polymyositis, immune-mediated necrotizing myopathy (IMNM), inclusion body myositis and myositis as part of an overlap syndrome [9]. IIM is rare disease with a prevalence of about 20/100 000 [10]. Inflammation in the skeletal muscles causes characteristic proximal muscle weakness. Extra muscular manifestations are common in IIM, including interstitial lung disease, dysphagia or arthritis and distinctive skin rash in dermatomyositis [10]. IIM is often associated with malignancy [11], however, molecular link explaining the increased risk of cancer in myositis patients is still poorly understood [12]. Autoantibodies can be detected in more than 80% of IIM patients, their occurrence is linked with characteristic syndromes as well as with specific HLA-haplotypes [13]. Information about the antibody positivity can be important for the choice of clinical treatment [14]. IIM are traditionally treated with high doses of glucocorticoids (GC), combinations of GC with GC-sparing immunosuppressive drugs are preferred nowadays. Discontinuation of GC may lead to a relapse. From the biologic drugs, so far only rituximab (anti-CD20 antibody) was shown to be effective in some cases of IIM, particularly in those with autoantibody positivity [10]. Similarly to RA, new therapeutic targets and/or biomarkers would improve the outcomes of IIM treatment.

The small regulatory RNAs are defined by their length (20-32 nt) and the interaction with the Argonaute family of proteins. I have focused on two classes of small regulatory RNAs: microRNAs (miRNAs) and PIWI-interacting RNA (piRNA) [15]. miRNAs (19-24 nt) in complex with Ago proteins regulate the gene expression of the majority of protein coding genes by cleaving the target mRNA or by blocking the translation [16]. Several miRNAs (miR-124a, -146a, -155 and -203) are deregulated in RA SF, synovial fluid and/or in the circulation, and are studied as a potential biomarkers in RA [17]. piRNAs are a bit longer than miRNAs (24-32 nt) and build complexes with another class of Argonaute proteins called PIWIL proteins (P-element induced wimpy testis like) [18]. PIWI/piRNA complexes regulate gene expression through target RNA degradation or the recruitment of chromatin modifying enzymes [18, 19]. While miRNAs and Ago proteins are expressed across the tissues, PIWI-proteins and piRNAs were considered to be restricted to germline and cancer [15]. However, recent studies have shown the possible role of piRNAs in somatic cells including regulation of protein coding genes [18, 20]. To our knowledge, piRNA expression in RA has not been studied so far.

S100 protein family consists of 21 small (≈10 kDa) calcium binding proteins, which are soluble in 100% ammonium sulphate (this gave them the name S100). Intracellularly S100 proteins act as calcium sensors, while extracellularly they are ligands of many receptors including TLR4, IL10 and G-protein coupled receptors [21]. S100 proteins contribute to growth and metastasis in cancer and first anti-S100 treatments are tested in clinical trials now [22]. In RA, several S100 proteins including S100A4, S100A8/9, and S100A12 are increased both locally in the synovial tissues or fluids and in the circulation [23-25]. Serum levels of S100A4 and S100A8/9 correlate with the disease activity in RA [26, 27]. In IIM, immunohistochemical staining showed presence of S100 proteins in the muscle biopsies [28].

Autoantibodies are immunoglobulins specifically recognizing antigenic structures on own tissues (autoantigens). Rheumatoid factors (typically IgM targeting Fc-fragment from IgG) and autoantibodies against citrullinated peptides are present in 50-80% of RA patients [1], while in IIM, about 80% of patients are positive for various myositis specific or myositis associated autoantibodies [13]. In both RA and IIM the autoantibodies positivity is associated with HLA-haplotype and more importantly, also with the clinical symptoms, the course of the disease and the response to the treatment [29].

2. AIMS OF THE THESIS

The aim of my PhD. thesis was to improve our understanding of pathogenesis and development of RA and IIM. I have focused on three molecular aspects: the role of small regulatory RNAs, S100 proteins and autoantibodies.

1) Role of small regulatory RNAs

   - Are PIWI-proteins and piRNAs expressed in the synovial fibroblasts?
     If so, is their expression different in RA then in osteoarthritis?
     How are the PIWI proteins and piRNAs regulated? What is their function?

   - Which microRNAs are deregulated in IIM?
     Are there any microRNAs associated with the disease activity in IIM?

2) Role of S100 proteins

   - Are the S100A4 serum levels increased in patients with IIM?
     Do the S100A4 serum levels correlate with the disease activity of IIM?
     What role does S100A4 (called metastasin) play in the cancer associated myositis?

   - What are the S100A8/9 serum levels in patients with a recent onset RA?
     Do the S100A8/9 serum levels correlate with disease activity in recent onset RA?
     How are the S100A8/9 serum levels changed by the treatment of RA?

3) Role of autoantibodies

   - Are the autoantibodies associated with arthritis development in the IIM?
     What are the general characteristics (prevalence, genetic associations, distribution in the joints, extent and severity) of arthritis as part of IIM?

   - How important are the various autoantibodies in the IMNM?
     Are the autoantibodies associated with the use of statins?
     Has the incidence of IMNM increased in the last 10 years?

3. METHODOLOGY

3.1. General description of methods

Patients

In our studies on RA, we included patients who fulfilled ACR/EULAR 2010 criteria for RA unless stated otherwise [30]. In studies on IIM, patients who fulfilled the Bohan and Peter myositis criteria [31, 32] or European Neuromuscular Centre criteria for necrotizing myopathy [33] or Griggs criteria for IBM [34] were included. If cancer occurred within 3 years of the diagnosis of myositis, we classified the IIM as cancer associated myositis (CAM). All individuals gave informed consent to participate and the study was approved by the local Ethics Committee.

Disease activity assessment

Activity of RA was assessed by disease activity score for 28 joints (DAS28) [35] and Swollen joint count for 66 joints (SJC) [36]. Disease activity of IIM was evaluated by MYOsitis disease ACTivity assessment (MYOACT) and Physician Global Activity using visual analogue scales (VAS), Manual Muscle Testing (MMT) and Health Assessment Questionnaire (HAQ) [37].

Laboratory measures

Serum levels of CRP, anti-cyclic citrullinated peptide antibodies (anti-CCP) and rheumatoid factors (RF) were measured by established laboratory techniques in all RA patients. All IIM patients were routinely assessed for serum levels of C-reactive protein (CRP) and the muscle-associated enzymes (creatinine kinase CK, lactate dehydrogenase LD). The autoantibody profiles were determined using IIF to screen for ANA and anti-dsDNA, line immuno-assay and myositis-western blot. In-house 35S radioimmunoprecipitation [38] was used to confirm the results and to detect autoantibodies not captured using commercial assays.

Statistical analysis

A Kolmogorov-Smirnov test of normality was performed for all variables and their difference scores. Pearson's product-moment correlation coefficients and Spearman's rank correlation coefficients were used in cases of normal and non-normal variables, respectively. When comparing patients and controls, the independent samples t-test was used for normal variables and the Mann-Whitney U test was used as a nonparametric alternative. For all statistical evaluations, P values below 0.05 were considered statistically significant. The statistical analyses were in some studies[1] performed in collaboration with a biostatistician.

[1] PIWI/piRNA in RA, both studies on S100 proteins

3.2. PIWI/piRNA system in RASF

Patients, synovial tissues and synovial fibroblasts cell culture

Synovial tissues were obtained from patients with rheumatoid arthritis diagnosed according to the 1987 American College of Rheumatology criteria for classification of RA [39] who underwent joint replacement surgery. Synovial tissues from patients with osteoarthritis (degenerative disease) served as a controls. Synovial fibroblast (SF) cultures were established by dispase digestion [40]. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Passages 4-8 were used for all experiments. RASF and OASF (n=5-12) were either stimulated or not for 24 hours with Poly(I:C) (10ug/ml), LPS (100ng/ml) or TNFα (10 or 100ng/ml) alone or in combination with IL-1β (1ng/ml).

RNA isolation, reverse transcription and real time polymerase chain reaction (RT-PCR)

Synovial tissues or cultured SF were lysed in QIAzol lysis reagent, and total RNA was isolated using a miRNeasy Mini kit. Total RNA was reverse transcribed using random hexamers and MultiScribe reverse transcriptase as recommended by the manufacturer. PCR was performed with the 7500 Real-Time PCR System. Relative expression was calculated by the comparative threshold cycle method, where dCt = Ct (mRNA of interest) − Ct (housekeeping gene).

Small RNA sequencing

Total RNA from RASF and OASF (n=9 each) was isolated as described above. RNA quality control, small RNA library preparations and sequencing using HiSeq2500 were performed at Functional Genomic Centre Zurich. The small RNA-seq reads were mapped to the human genome and aligned to piRNABank – a database of 19 260 human piRNA sequences [41]. In RA versus in OA identified piRNAs were compared using SARTools with edgeR package [42].

Silencing of PIWIL4

RASFs were transfected with small interfering RNAs (siRNA) targeting PIWIL4 using Lipofectamine 2000. Knockdown was verified by Real-time PCR and Western blot.

Western bloting

Nuclear and cytoplasmic extracts from SFs were prepared with NC-PER Nuclear and Cytoplasmic Extraction Reagents. Samples in Laemmli Buffer were separated on 10% SDS–polyacrylamide gels and electroblotted on polyvinylidene fluoride (PVDF) membrane. After blocking, the membranes were probed with rabbit anti-human PIWIL4 or PIWIL2 antibodies, incubated overnight, washed and incubated with secondary HRP-conjugated goat anti-rabbit IgG. Signals were detected using Western BrightTM ECL and the Fusion FX imager. Lamin B1 and Tubulin were used as the internal controls.

Functional assays

Apoptosis was measured with the Annexin V/PI assay according to the manufacturer's instructions. Proliferation was analysed with the xCELLigence system, which enables the real-time analysis of cell adhesion and proliferation by measurements of electric impedance. [43]. With Global Methylation LINE-1 kit we determined the methylation of LINE-1.

Statistical analysis

Data were analysed by parametric (paired 2-tailed t-test) or nonparametric (Wilcoxon's matched pairs signed rank test) statistical tests as appropriate. Values are presented as mean ± SD. For statistical analysis, GraphPad Prism 5.0 software was used.

3.3. Circulating microRNAs in IIM

Patients and samples Peripheral blood samples were obtained from 28 patients with IIM and controls (16 healthy individuals and 16 patients with systemic lupus erythematosus (SLE)).

RNA extraction, reverse transcription and miRNA microarray Total RNA was isolated from patient´s sera with phenol-chlorophorm extraction according to Filkova et al [44]. 300ng of RNA were transcribed into cDNA, labelled with Cy3-pCp and hybridized on the microarray. After washing, Cy3 was detected by DNA microarray scanner type G2502B and the data extracted with Feature Extraction Software.

Statistical analysis The array data were normalised by averaging summarisation of global medians across all arrays. The Student´s t-test was used to identify differentially expressed miRNAs. Arraystare software was used for the analysis.

3.4. S100A4 in IIM

Patients and laboratory measurements A total of 104 patients with IIM (43 DM, 39 PM and 22 CAM) and 77 healthy controls were enrolled in the study. Serum S100A4 concentrations were measured by ELISA according to the manufacturer´s protocol [26].

Statistical analyses The data were described as median (IQR) if variables were not normally distributed and as mean (SD) if normally distributed. The Kruskal-Wallis test and corresponding post-hoc analysis and Mann-Whitney test were conducted for comparison between groups. Before analysis of associations among variables, the data were first normalitized. The analysis was performed using SPSS 17.0 and the graphs were prepared by GraphPad Prism.

3.5. S100A8/9 and S100A12 in recent-onset RA

Patients and clinical examination A total of 43 patients with recent onset RA were enrolled in the study. Symptom duration was less than 6 months and patients were treatment naïve at baseline. Disease activity was assessed at the baseline and after 3 months of conventional treatment. Control group consisted of 32 healthy individuals. Serum S100A8/9, S100A12 were detected by ELISA Kit according to the manufacturer´s protocol.

Statistical analysis The concentrations of S100 proteins are expressed as means ± SEM. Statistical analyses were performed using SPSS software version 17.

3.6. Arthritis in IIM: clinical features and autoantibodies

Patients All patients with IIM seen both at the outpatient and inpatient departments of the Institute of Rheumatology between January and September 2012 were recruited into the study. All patients had a muscle biopsy performed during the course of their disease. Radiographs of the joints of hands and/or feet were available from 47 patients. Information regarding presence or absence of arthritis in the past and/or at the current time with respect to the onset, localization, and symmetry was obtained during personal interviews with patients and/or from medical records. .

Statistical analysis The continuous not-normally distributed variables were analysed by a Mann-Whitney test; categorical data were analysed by Fisher's exact test having p values estimated by Monte Carlo simulations (n = 10.000), and Kaplan-Meier estimator was used for calculation of survival analysis of arthritis. The significance of differences in allele and gene frequencies was evaluated by Fisher's exact test. We used GraphPad Prism 5 and R (r-project.org) for statistical analyses.

3.7. Immune-mediated necrotizing myopathy: incidence and autoantibodies

Patients and samples Clinical data and muscle biopsies of all patients who were referred to the Institute of Rheumatology between January 2004 and June 2014 for suspicion of IIM were retrospectively reviewed. Biopsy results were subcategorized according to the 119th ENMC workshop [33].

Autoantibodies Anti-HMGCR autoantibodies were measured by ELISA in 218 patients and in 62 healthy controls according to manufacturer's instructions. Negative cut-offs were calculated from the mean optical density + 3 SD of healthy controls

Statistical analysis Categorical data were analysed by $\chi 2$ test and Fisher's exact test. We used GraphPad Prism 5 for statistical analysis.

4. RESULTS

4.1. Small regulatory RNAs

4.1.1. PIWI/piRNA system in RASF

PIWIL2 and 4 are expressed in RA and OA synovial fibroblasts

PIWI proteins are binding partners of the PIWI-interacting RNAs and were initially believed to be expressed in the germline-cells and cancer [15].

To investigate if PIWI/piRNA system is active in the synovial cells, we first measured the expression of the PIWIL1-4 mRNA in RA and OA synovial tissues. We found mRNA for PIWIL2 and PIWIL4 to be expressed at similar levels in both RA and OA synovial tissues, while mRNA for PIWIL1 and PIWIL3 was not detectable (Figure 1A).

Synovial tissue is composed of multiple cell types (synovial fibroblasts, inflammatory infiltrates, endothelial cells and others), and synovial fibroblasts themselves are recognized as key players in RA pathogenesis [45]. We measured the expression of PIWIL1-4 mRNA in cultured synovial fibroblasts and detected a similarly high expression of PIWIL2 and PIWIL4 mRNA but not PIWIL1 and PIWIL3 (Figure 1B).

On the protein level, we detected strong expression of PIWIL4 in nuclear extracts from RASF and OASF on Western blots, while the bands of PIWIL2 were weak in both RASF and OASF. There was no PIWIL2 or PIWIL4 protein in the cytoplasmic extract (Figure 1C).

For the following analysis, we focused on PIWIL4 and performed immunohistochemistry in synovial tissues, where we observed a strong signal in both, RA and OA tissues (Figure 1D).

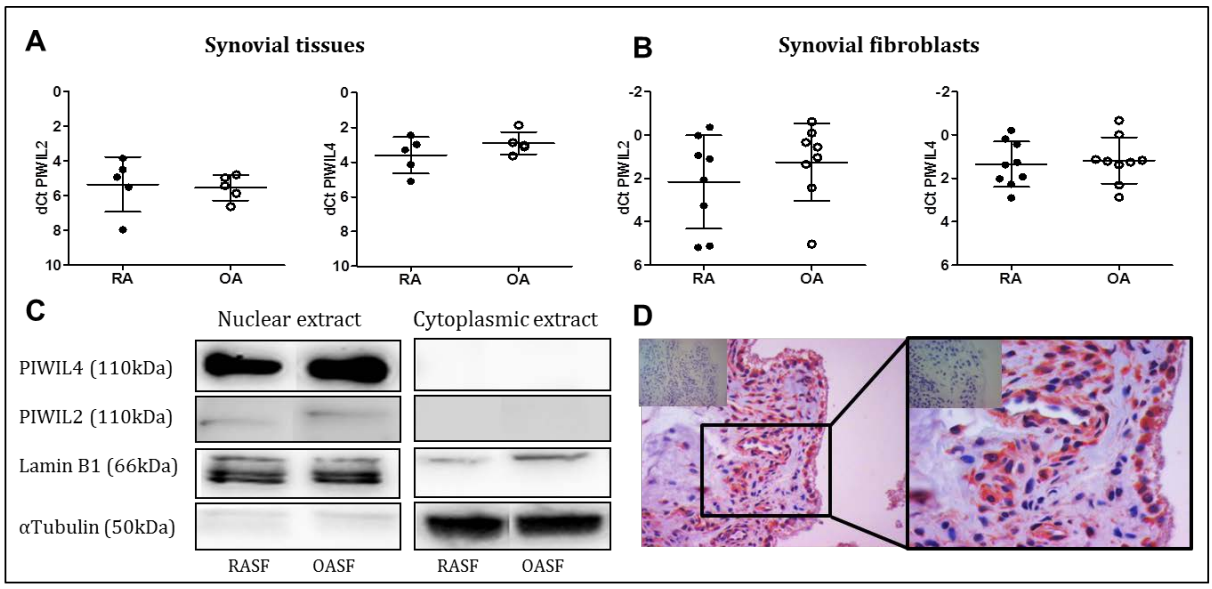

Figure 1 PIWIL2 and 4 are expressed in RA and OA synovial tissues and synovial fibroblasts

RASF express 267 PIWI-interacting RNAs; piR-823, -4153 and -16659 are deregulated in RASF when compared to OASF

Next we sequenced 9 RASF and 9 OASF small RNA libraries using the Illumina platform. The length distribution of the reads peaked at 22 nt and 29 nt, confirming the presence of both miRNAs and piRNAs (Figure 2A1).

In all 9 RASF there were 267 piRNAs detected, while in OASF 244 piRNAs were detected. Unsupervised clustering of piRNA could not distinguish between the groups of RASF and OASF (Figure 2A2). The analysis of RNA seq count data by the edgeR did not show any differentially expressed piRNAs in RASF compared to OASF.

Expression levels of single piRNAs do positively correlate with each other. Figure 2B1 presents the correlation coefficients R between 38 piRNAs (only the most expressed once, with mean RPKM ≥500) and 2 endogenous controls in the 9 RASF and 9 OASF. The positive correlations (R≥0.5) are highlighted in orange. piRNAs mostly do not correlate with the endogenous controls RNU-6B showing, that the positive correlation is not a result of unequal read counts within the samples.

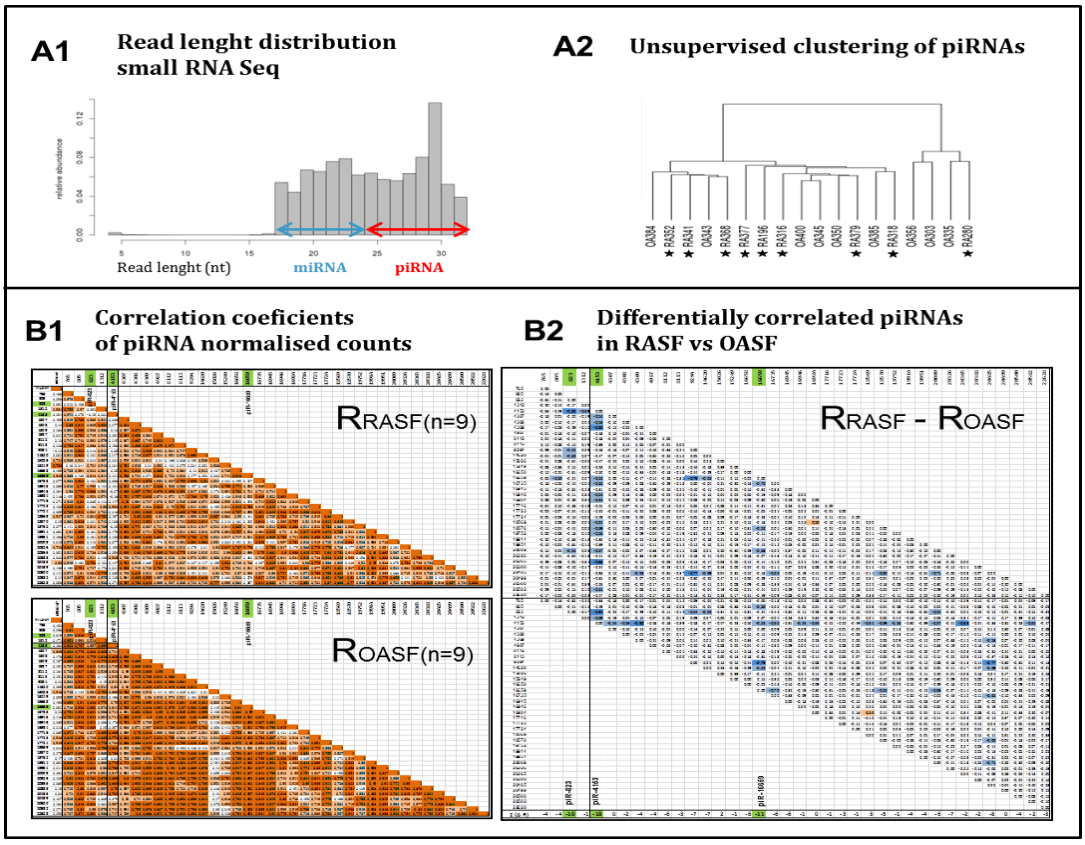

Figure 2 RASF express 256 PIWI-interacting RNAs; piR-823, -4153 and -16659 are deregulated in RASF when compared to OASF

The coexpression/coregulation of piRNAs seemed to be less tight in RASF compared to OASF (the correlation matrix from RASF is less orange than the one from OASF). To confirm our visual impression, we calculated the ΔR=R(RASF)-R(OASF) and then ΣΔR for each piRNA. ΔR were mostly negative as shown in the Figure 2B2 and the 3 most deregulated piRNAs in RASF were piR-4153, -16659 and -823 (highlighted in red).

Stimulation with TNFα+IL-1β, LPS or Poly(I:C) upregulates PIWIL4 expression and deregulates piRNA coexpression

To understand, if PIWIL4 is regulated by the inflammation process in the joint, we measured PIWIL4 expression in RASF and OASF stimulated with TLR-ligands LPS and Poly(I:C) or with proinflammatory cytokines IL-1β in combination with TNFα. Levels of PIWIL4 mRNA were enhanced by Poly(I:C) in both RASF and OASF 2.9-fold (p=0.003)/3.4-fold (p=0.013); LPS 2.1-fold (p=0.026)/2.6-fold (p=0.025) and TNFα in combination with IL-1β 1.9-fold (p=0.003)/1.7-fold (p=0.007) (Figure 3A). On the protein level we detected by Western blot similar induction of PIWIL4 expression in the nuclear extract, while in the cytoplasmic extract PIWIL4 protein was not detectable (Figure 3B).

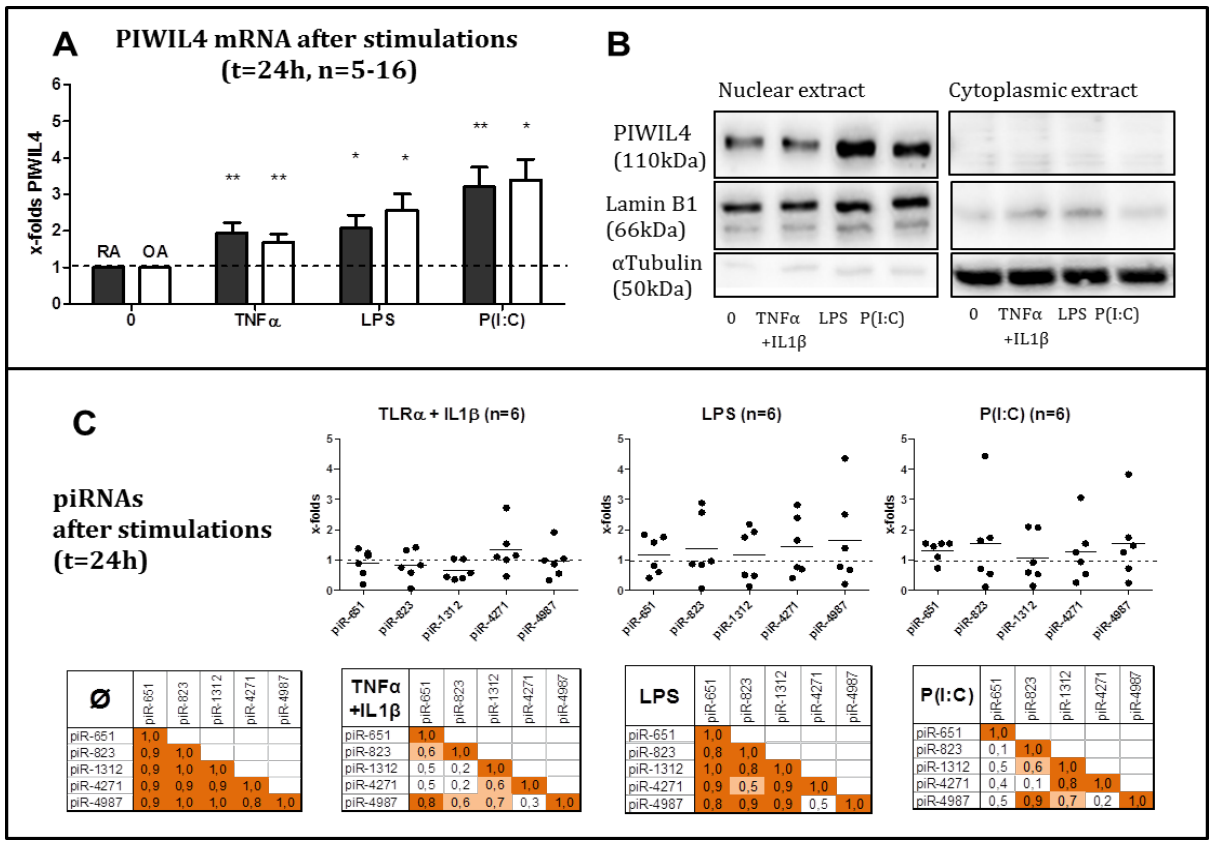

Figure 3 Stimulation with TNFa+IL1b, LPS or POLY(I:C) upregulates PIWIL4 expression and deregulates piRNAs coexpression

The expression of piR-651, -823, -4987, -1312 and -4271 in RASF was not significantly changed 24 hours after stimulation with TNFα + IL-1β, LPS or Poly(I:C), but their coexpression became deregulated after TNFα/IL-1β or Poly(I:C) (Figure 3C). Correlation coefficients of piRNA x-folds are graphed in heat maps. Strong positive correlations (0.75≤R) are highlighted with dark orange; weak positive correlations (0.5≤R<0.75) are light orange and no correlation (-0.5≤R<0.5) are white.

Silencing of PIWIL4 decreases HDAC1 expression and increases TNF-α-induced apoptosis in RASF

To study the function of the PIWI/piRNA in SF, we have silenced the PIWIL4 gene. PIWIL4 silencing efficiency was 50% on the mRNA level and 30% on the protein level. We have not observed any changes in piRNA levels after silencing of PIWIL4 (Data not shown).

PIWI/piRNA-complexes recruit epigenetic modifiers on the target place [18]. We questioned whether PIWIL4 silencing had any impact on expression of HDAC 1-11 in RASF. 48 hours after siPIWIL4, we have observed a decreased mRNA expression of HDAC1 (to 0.6-fold, p=0.0003) and an increased expression of HDAC9 (to 1.3-fold, p=0.025), while levels of other HDAC remained unchanged (Figure 4C). However, there was no change in HDAC1 protein level measured 72 hours after silencing PIWIL4 (data not shown)α.

Because in cancer the overexpression of PIWIL4 decreases apoptosis [46] and the reduced apoptosis susceptibility contributes to the synovial hyperplasia in RA [45], we evaluated the cell apoptosis in RASF and OASF (n=5) silenced for PIWIL2 and PIWIL4 genes after high dose of TNFα (100ng/ml). Silencing of PIWIL genes combined with TNFα stimulation increased the apoptosis compared to TNFα stimulation alone (RASF: 1.5-fold, p=0.050; OASF: 1.2-fold, p=0.639) (Figure 4E). Silencing of PIWIL4 did not change cell proliferation or the expression of the LINE-1 retrotranspozome (data not shown).

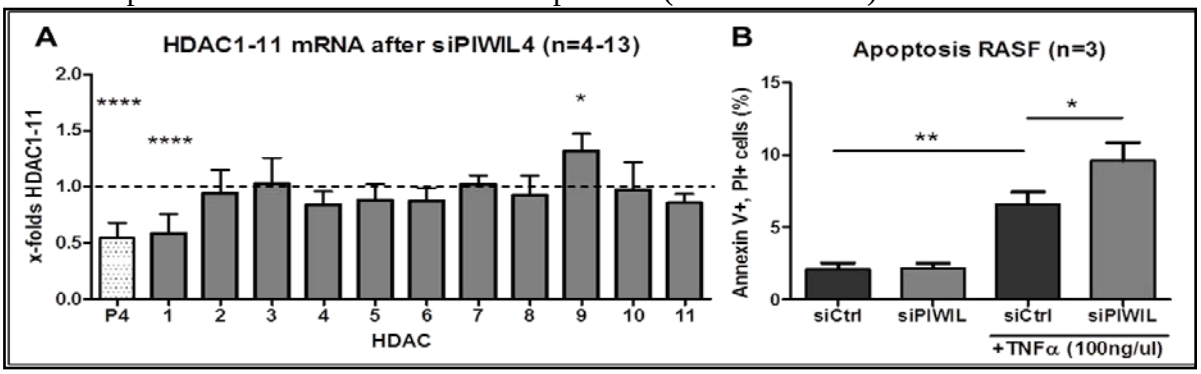

Figure 4 Silencing of PIWIL4 decreases HDAC1 expression and increases TNFα-induced apoptosis in SF

4.1.2. Circulating miRNAs in IIM

Six microRNAs are deregulated in sera from IIM patients compared to healthy controls

With the microRNA microarray method we have determined expression of 2 549 circulating miRNAs (c-miRNAs) in the sera of 28 patients with IIM and 16 healthy controls (HC). We found six c-miRNAs to be significantly deregulated in IIM patients compared to HC (Table 1). let-7b*, miR-498 and miR-4310 were upregulated, while miR-1234, miR-3679-5p and miR-4299 were downregulated in IIM compared to HC.

SystematicName	Myositis	Controls	Fold change	*p*-value
hsa-miR-1234	11,822	14,659	-1,24	0,0078
hsa-miR-498	14,159	9,328	1,517	0,0113
hsa-miR-3679-5p	141,668	307,403	-2,169	0,0152
hsa-miR-4299	22,444	74,922	-3,338	0,0255
hsa-let-7b*	5,874	4,251	1,381	0,0412
hsa-miR-4310	6,542	4,537	1,442	0,0451

Table 1 Differentially expressed miRNAs in sera from patients with IIM compared to healthy controls

miR-877, -3676 and -3907 were overexpressed in IIM patients with high disease activity

Further we have studied the associations of c-miRNAs expression with IIM disease activity. Three miRNAs (miR-877*, -3676 and -3907) were upregulated in patients with high disease activity (DA) compared to patients with low DA (Figure 5).

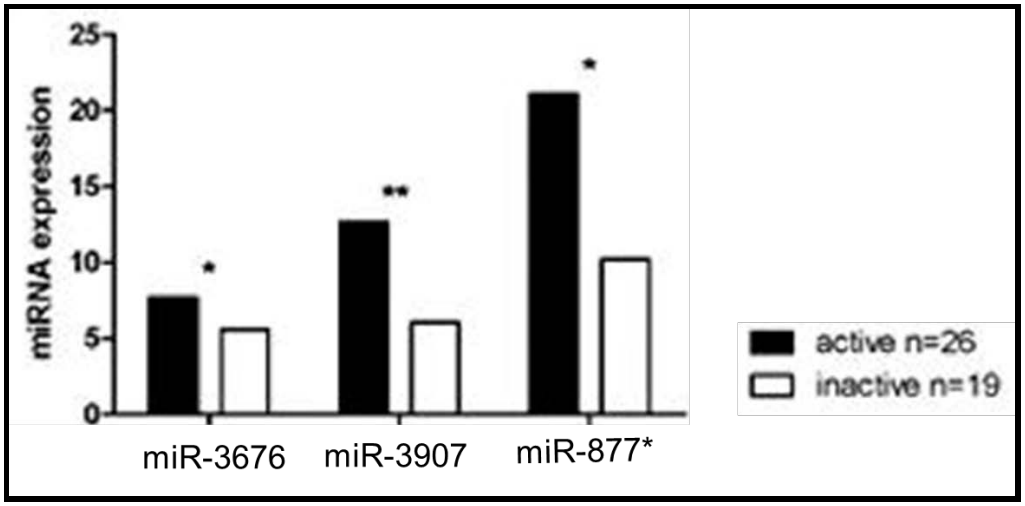

Figure 5 Three miRNAs were differentially expressed in patients with high disease activity (DA) compared to patients with low DA

4.2. S100 proteins

4.2.1. S100A4 in IIM

Circulating S100A4 is elevated in polymyositis

S100A4 protein (metastasin) is known for its contribution to the metastatic process in cancer, but its expression is also increased in several autoimmune diseases. Levels of circulating S100A4 in IIM have not been studied yet. This was surprising, because IIM are autoimmune diseases with a high risk of malignancy. First, we measured serum levels of S100A4 in 43 dermatomyositis (DM), 39 polymyositis (PM) and 22 cancer associated myositis (CAM) patients and in 77 HC by ELISA. Circulating S100A4 was significantly higher in PM patients compared to HC or DM patients. Serum S100A4 levels in CAM were, to our surprise, not different from HC or DM and lower compared to PM (Figure 6).

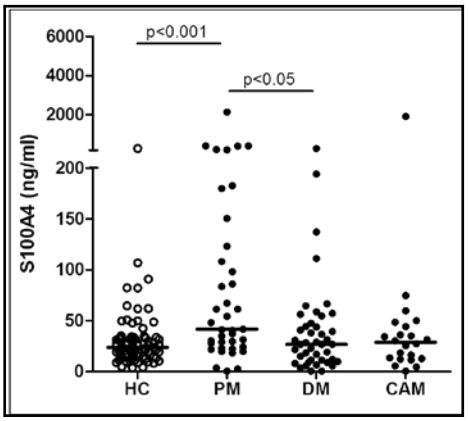

Figure 6 c-S100A4 is elevated in PM

Circulating S100A4 is lower in anti-TIF1-γ positive IIM

Next, we compared S100A4 levels in IIM patients with different autoantibodies. We observed a significant decrease of circulating S100A4 in anti-TIF1-γ positive patients (Figure 7). Transcriptional factor TIF1-γ is often targeted by autoantibodies in CAM, and IIM patients with anti-TIF1-γ autoantibodies have an increased risk of developing cancer. There are two possible explanations for low S100A4 levels in those patients: consumption of S100A4 in the tumour tissue or increased occurrence of S100A4 in the form of multimers caused by the cancer, which were not detected by our ELISA. Furthermore, we detected increased S100A4 serum levels in anti-Jo-1 and anti-PM-Scl positive patients. There was no difference in anti-Mi-2 positive patients and because of the low prevalence, we could not investigate S100A4 levels in the other autoantibodies (data not shown). IIM patients with anti-Jo-1 autoantibodies have an increased risk of arthritis and S100A4 is known to play role in rheumatoid arthritis, however when we compared S100A4 levels in patients with and without arthritis, we did not see any difference. We concluded that the pathogenesis of arthritis in IIM is probably different from RA.

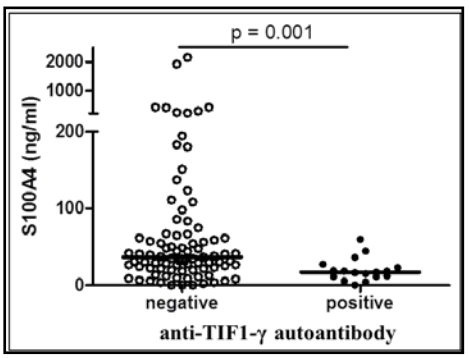

Figure 7 Circulating S100A4 is low in anti-TIF1-γ + IIM

Circulating S100A4 correlates with disease activity in IIM

Circulating S100A4 correlated moderately with MYOACT score and its multiple components (extramuscular disease activity (DA), pulmonary DA, constitutional DA) (Figure 8) as well as with the laboratory parameters CRP, CK and LD (data not shown). However, there was no correlation with the muscle DA, MMT result or with the findings in the muscle biopsy, suggesting that S100A4 is reflecting rather the global DA and systemic inflammation, than specifically the activity of the muscle involvement.

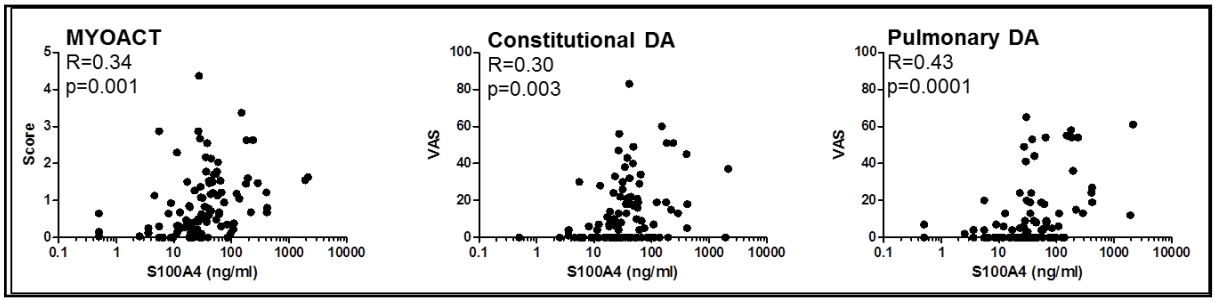

Figure 8 Circulating S100A4 correlates with disease activity in IIM

4.2.2. S100A8/9 and S100A12 in recent-onset RA

S100A8/9 and S100A12 are increased in recent-onset RA and normalise with treatment

S100A8/9 and S100A12 are proinflammatory S100 proteins, which are known to be increased in established rheumatoid arthritis both locally in the inflamed joints and in the circulation. We measured S100A8/9 and S100A12 proteins by ELISA in the sera of 43 patients with recent onset RA, who have not yet started the treatment with conventional DMARDs. This was done at baseline (first visit in our clinics) and then after 3 months of treatment. We compared these patients with 32 HC. A significant increase in both S100 proteins in the recent-onset RA patients was found and the levels normalised after the treatment (Figure 9).

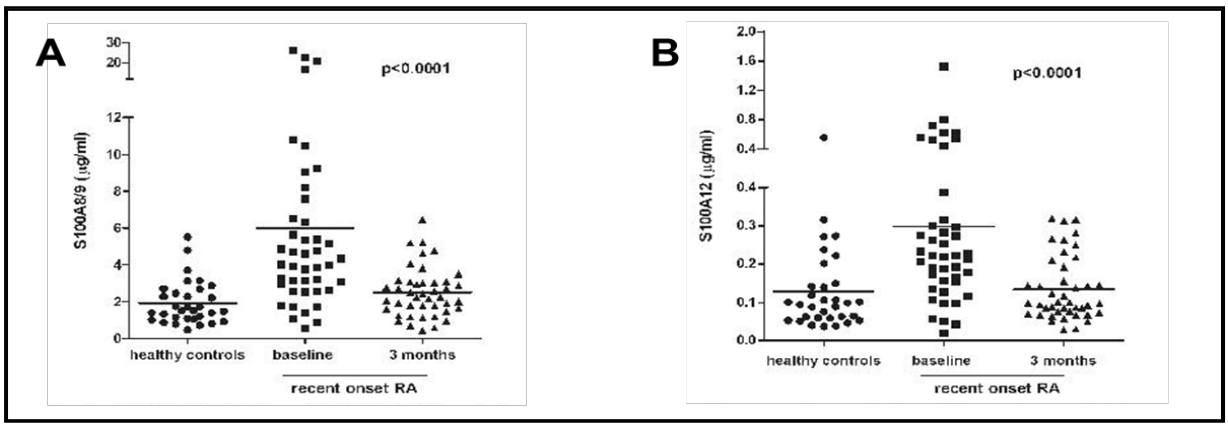

Figure 9 S100A8/9 **(A)** and S100A12 **(B)** are increased in recent-onset RA and normalise with treatment

Circulating S100A8/9 correlates with disease activity in early RA

Very early RA is an immunopathologically distinct phase of the disease where the "window of opportunity" for early treatment potentially preventing joint damage may exist. Our aim was to prove if S100A8/9 or S100A12 would be suitable as biomarkers in very early RA. Circulating S100A8/9 significantly correlated with all SJC (Figure 10A), DAS28 and CRP, correlations of S100A12 were less tight (data not shown). Further, the change in S100A8/9 levels during the 3 months of treatment correlated significantly with the changes of SJC (Figure 10B), DAS28 and CRP, while the change of S100A12 correlated only with the change in SJC (data not shown). The tight relation of circulating S100 proteins and SJC is indirectly confirming the hypothesis that these proteins are synthesized in the synovial membrane of inflamed joints and then released in the circulation.

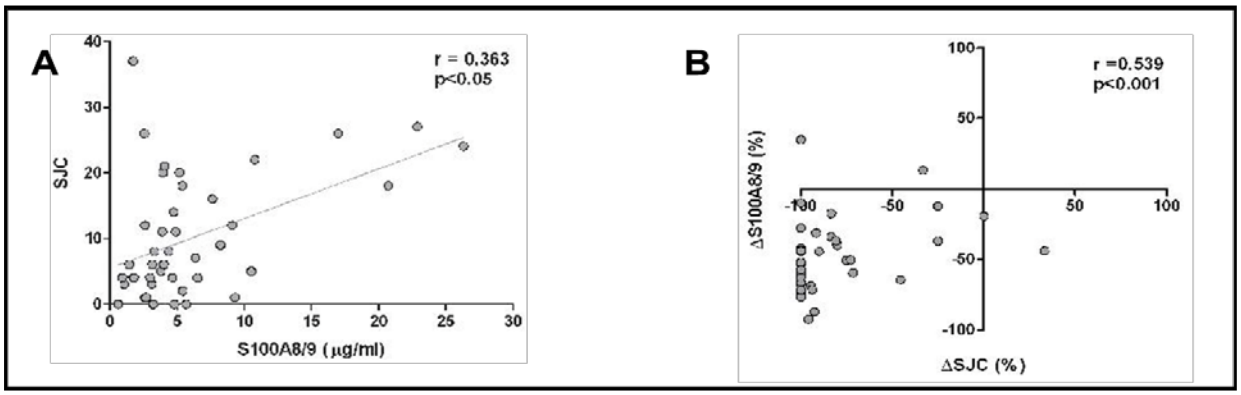

Figure 10 Circulating S100A8/9 **(A)** and its change **(B)** correlate with swollen joint count (SJC) and its change

Decreases in circulating S100A8/9 predict improvement in swollen joint count after 3 months of treatment in early RA

Results from the multiple linear regression suggest that S100A8/9 serum levels at the baseline are better predictors for SJC than S100A12 or CRP serum levels. In addition, change of S100A8/9 serum levels during the 3 months of treatment was the only predictor for change in SJC (Table 2).

Initial SJCs (n = 43, r^2 = 0.260, F = 8.360; P = 0.001)[b]					ΔSJCs (n = 41, r^2 = 0.245, F = 13.974; P = 0.001)[c]				
Variables	Parameter estimate	SEM	t-value	P value	Variable	Parameter estimate	SEM	t-value	P value
Intercept	5.093	1.712	2.975	0.005	Intercept	-5.192	1.148	-3.500	0.001
Initial CRP	0.147	0.071	2.067	0.045	ΔS100A8/9	0.001	0.000	3.738	0.001
Initial S100A8/9	0.001	0.000	2.010	0.051	-	-	-	-	-

[a]SJCs, swollen joint counts; SEM, standard error of the mean; CRP, C-reactive protein. r^2 values are adjusted. [b]Excluded predictors are age and initial S100A12. [c]Excluded predictors are age, initial S100A12, initial S100A8/9, initial CRP, ΔS100A12 and ΔCRP.

Table 2 Multiple linear regression models for initial SJC and change in SJC

4.3. Autoantibodies

4.3.1. Arthritis in IIM: clinical features and autoantibodies

Arthritis in IIM is associated with anti-Jo-1 positivity

In our study we determined the prevalence of arthritis in 106 unselected IIM patients and its association with autoantibody profiles. At the cross-sectional physical examination 29% of the patients presented with at least one swollen joint. The evaluation of the medical records revealed that 37% of patients had arthritis at the onset of IIM and in 53% of patients arthritis occurred at any time during the disease course (Table 3). Combining several autoantibody-testing methods including the radioimmunoprecipitation we detected autoantibodies in 82% of IIM patients. We have confirmed the strong association of anti-Jo-1 autoantibodies with arthritis, 93% of anti-Jo-1 positive patients experienced arthritis at any time during the disease, while this was the case for only 34% of anti-Jo-1 negative patients, this difference was significant ($p<0.0001$). No significant associations with arthritis were found for ANA, anti-Ro, anti-PM-Scl, anti-Mi-2 and anti-TIF1-γ. Other autoantibodies were found in too low frequencies to be statistically evaluated, however 7 of 9 patients positive for the rheumatoid factor and both patients with ACPA positivity had arthritis.

Arthritis in IIM is rarely deforming or erosive

In our cohort of IIM patients, arthritis most often affected wrists, shoulders and the small joints of the hands. This localisation reminds of rheumatoid arthritis. Joint symptoms can precede the muscle weakness, which happened in 59% of our patients who had arthritis at the disease onset. Arthritis in IIM is typically symmetrical nonerosive polyarthritis. The mean arthritis activity and joint damage scores in IIM are low due to high proportion of non-affected patients, however in IIM patients with joint involvement the mean joint disease activity and damage are moderate and higher scored by patients than by clinicians (Table 4).

Diagnosis	Arthritis at Any Time*	Arthritis at Disease Onset**	Current Arthritis# (≥ 1 swollen joint)
PM (46)	27 (59)	19 (41)	17 (40)
DM (40)	22 (55)	15 (38)	11 (28)
CAM (8)	2 (25)	1 (13)	0 (0)
IMNM (11)	4 (36)	1 (13)	2 (18)
IBM (1)	1 (100)	1 (100)	1 (100)
Total (106)	56 (53)	39 (37)	31 (29)

Table 3 Arthritis in myositis subtypes.
Data are in %. PM:polymyositis; DM:dermatomyositis; CAM: cancer associated myositis+ IMNM: immune mediated necrotizing myopathy; IBM: inclusion body myositis

	All Patients	Patients with VAS > 0
MD activity	7.5 ± 15.8	19.4 ± 20.5 (41)
MD damage	6.2 ± 15.6	21.2 ± 22.8 (31)
Pt activity	14.0 ± 21.5	26.5 ± 23.4 (56)

Table 4 Arthritis activity and damage. Data shown as mean ± SD (n)
MD/Pt: Physician´s/patient´s assessment of arthritis activity/damage on visual analog scale (VAS, 100mm)

4.3.2 Immune-mediated necrotizing myopathy: incidence and autoantibodies

Incidence of IMNM is increasing

IMNM is characterised by the presence of necrotic muscle fibres with minimal inflammatory infiltrates in muscle biopsy. The muscle weakness is usually severe and the response to immunosuppressive treatment variable. The diagnosis of IMNM seemed to be becoming more frequent in our centre, therefore we designed a retrospective analysis of the annual incidence of IMNM in 2004 - 2014. During this period in total 233 patients were diagnosed with inflammatory/immune-mediated myopathy in the Prague Institute of Rheumatology. Based on the muscle biopsy evaluation, 39% of those patients had DM, 28% had PM, and 12% had IMNM. The remaining biopsies were unclassifiable/with non-specific findings or normal. We have confirmed the increased incidence of IMNM: Figure 11 illustrates that in years 2004-2007 there was no case of IMNM, during the following 4 years IMNM incidence was under 10% of all IMNM and in the last 2 years of the study, IMNM makes about ¼ of all IIM.

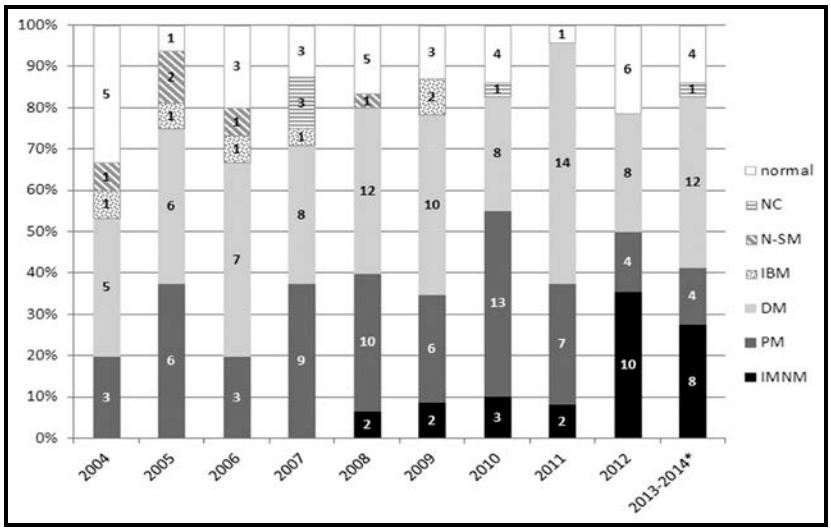

Figure 11 Percentage distribution of biopsy results in 2004-6/2014

Anti-HMGCR positive statin related IMNM

Sera from all IIM patients were tested for the presence of myositis-specific and -associated autoantibodies with several methods including the radioimmunoprecipitation. Out of the 27 patients with IMNM, 11 were anti-HMGCR positive. Only four patients who were anti-HMGCR positive did not have IMNM (based on the muscle biopsy they were classified as PM). Further autoantibodies detected in IMNM patients were anti-SRP, anti-Jo-1, anti-Ro/La, anti-CCP, anti-Ku, anti-Ro52 and ANA. All 15 anti-HMGCR positive patients had the history of statin treatment, while only 21 out of the 202 anti-HMGCR negative patients (10%) had been treated with statins ($p<0.0001$). Our data show that the increased incidence of IMNM is accounted by anti-HMGCR positive patients with the history of statin usage.

5. DISCUSSION

5.1. Small regulatory RNAs

PIWI-interacting RNAs and PIWI proteins were discovered in germline cells [47] and initially believed to play a role only in the germline-cells and cancer [15, 48], but recent studies analysed the presence of PIWIL4 and piRNAs in somatic tissues as well.[20] We describe the expression of piRNAs in synovial fibroblasts (SF). The number of piRNAs which we detected in RASF (267 piRNAs) is comparable with 273 piRNAs detected in somatic tissues by Martinez et al [49]. Further we have shown that piRNAs in SF are most likely co-regulated by some common mechanism, as their expression levels do correlate positively with each other. This regulation is weaker in RASF than in OASF, and particularly piR-4153, piR-16659 and piR-823 seem to be deregulated in RASF. Further studies are needed to clarify whether the piRNA deregulation is deeper involved in the activation of RASF or it is its bystanding effect. The PIWI/piRNA system is known to be regulated by sexual hormones [50, 51]; in addition, we are reporting upregulation of PIWIL4 expression on both mRNA and protein level by proinflammatory cytokines and TLR-ligands. This regulation suggests a possible involvement of the PIWI/piRNA axis in inflammation. It might be interesting to investigate if the PIWI/piRNA system is regulated by glucocorticoids, as these steroids are chemically related to sexual hormones and often used in treatment of patients with arthritis.

In our second project we studied c-miRNAs in IIM [52]. Changed expression patters of c-miRNAs were described in several autoimmune diseases including SLE, RA, Scl or vasculitis [53, 54]. In IIM only small studies with preselected candidate c-miRNAs were performed so far [55, 56], while our array screened levels of 2 549 c-miRNAs, which we measured in 28 IIM patients and 16 healthy controls. We detected 6 c-miRNAs to be deregulated in IIM patients, expression of miR-1234, -3679-5p and -4299 were decreased, while let-7b, miR-498 and miR-4310 were upregulated in the IIM patients compared to healthy controls. Let-7b was suggested as a biomarker for myocardial infection and is also increased in cardiovascular conditions such as heart hypertrophy, cardiac fibrosis, dilated cardiomyopathy, arrhythmia, angiogenesis, atherosclerosis, and hypertension [57]. Cardiovascular manifestations constitute a major cause of death in myositis [58], so further focused study of let-7b in IIM would be of interest.

5.2. S100 proteins

In our study on S100A4 in IIM [59] we demonstrated 1) increased S100A4 serum levels in patients with polymyositis, 2) relation between S100A4 and anti-TIF1-γ, anti-Jo1 and anti-PM/Scl autoantibodies and 3) association between S100A4 levels and several features of myositis disease activity, particularly with extramuscular symptoms.

S100A4 protein is for its cancer-promoting properties [60-62] also called metastasin and given the association of myositis with increased risk of cancer development [12, 63], we were surprised not to see increased S100A4 levels in cancer associated myositis. We can speculate that either there was S100A4 consumption in cancer tissue or inability to detect enhanced formation of S100A4 multimeric forms that occur during malignancies [64, 65]. Positive correlation between circulating S100A4 and disease activity has been already shown in RA [26], now we describe similar association in IIM. Interestingly circulating S100A4 levels correlate particularly well with extramuscular disease activity measures, but were not associated with muscle disease activity score in manual muscle test or levels of muscle enzymes. These results are consistent with the findings showing that muscle fibres do not significantly contribute to S100A4 production and myocytes do not respond to stimulation with S100A4 protein by production of pro-inflammatory cytokines [66, 67]. Thus, we can speculate that circulating S100A4 protein may reflect the global disease activity, including extramuscular organ involvement, rather than functional muscle impairment in inflammatory myopathies. In this context, association between S100A4 levels and pulmonary disease activity may be of clinical significance. However, these results should be confirmed in further targeted studies.

S100A8/9 and S100A12 proteins act as alarmins signalling tissue damage or inflammation [68]. We have measured circulating S100A8/9 and S100A12 in patients with recent onset RA before they were given an immunosuppressive treatment, which can skew the results [69]. In agreement with previous studies on established RA [70] we describe an increase of both alarmins and their association with laboratory and clinical markers of disease activity in recent-onset RA patients. S100A8/9 correlates better with various clinical and laboratory parameters than S100A12. Change in S100A8/9 but not in CRP is predicting change in swollen joint counts. This data support the hypothesis that S100A8/9 is largely produced by activated immune cells in the synovial membrane and pass into the circulation [71]. S100A8/9 protein is good candidate biomarker in both established and recent onset RA providing valuable information about the extent of local inflammation in the affected joints.

5.3. Autoantibodies

Autoantibodies are detectable in more than 80% patients with IIM, and they are very useful markers for clinical diagnosis, classification and for predicting the prognosis of IIM [72]. Patients with the same autoantibody are likely to share the HLA haplotype as well as clinical symptoms of IIM and responseto the treatment [29].

In our comprehensive overview of arthritis in an unselected cohort of IIM patients, we confirmed a strong association between anti-Jo-1 autoantibody positivity and presence of arthritis [73]. The prevalence of arthritis in anti-Jo-1 positive patients was in our cohort even stronger (93%) than in previous studies [74, 75]. Furthermore, we found that arthritis is affecting each second IIM patient at some point in the disease. Arthritis is often present at the beginning of the disease, in 22% of patients being the very first symptom of myositis. Most frequently involved joints according to our analysis are small joints on the hands and shoulders, which in absence of other IIM symptoms can lead to misdiagnosis of RA [76]. Data on the arthritis at disease onset were collected in a retrospective manner, which could have lead to the arthritis overestimation. However, in the crossectional assessment, almost 30% of IIM patients presented clinically apparent arthritis. Classification criteria for RA fulfilled only one IIM patient. This is in contrast with reports describing relatively common RA x IIM overlap syndrome [77]. Radiographs of our patients were showing mostly nonerosive arthritis and clinically the arthritis was considered by both patients and physicians mostly as low to medium active.

In the study on immune-mediated necrotizing myopathy (IMNM) we report increasing incidence of this subtype of IIM among our IIM patients over 10 years (2004-2014) [78]. We confirmed, that anamnesis of statin treatment is linked with the presence of autoantibodies against 3-hydroxy-3-methylglutaryl-Coenzyme A (anti-HMGCR) in IMNM patients [79]. Out of the 27 IMNM patients, 13 have history of statin use and 11 of those are anti-HMGCR positive. None of the statin non-users in the whole IIM cohort has anti-HMGCR positivity. Skeletal muscle-specific HMGCR knockout mice model of necrotising myopathy developed by Osaki et al [80] will hopefully help to investigate the molecular background of statin induced IMNM. Cardiovascular diseases are the leading cause of death and statin treatment is very efficient in their prevention of fatal events [81], therefore improving their safety would be of great interest.

6. CONCLUSION

In the studies included in this PhD thesis we demonstrated the importance of three different molecular aspects (regulatory RNAs, S100 proteins and autoantibodies) in systemic autoimmune diseases.

For the first time we characterised PIWI-interacting RNAs in rheumatoid arthritis (RA) synovial fibroblasts and screened circulating microRNAs in idiopathic inflammatory myopathies (IIM). We have analysed the clinical significance of circulating S100A4 in IIM and S100A8/9 and S100A12 in recent onset RA. Furthermore, we evaluated the prevalence of arthritis in IIM and confirmed its strong association with anti-Jo1 autoantibodies. We also indirectly linked the increase in IMNM to the use of statins causing formation of anti-HGMCR autoantibodies.

The pathogenesis of systemic autoimmune diseases is very complex and we have just added tiny fragments to the big puzzle. Understanding better this puzzle should improve patient care by the use of specific biomarkers followed by personalised treatment with the drugs targeting specific targets coming in the future.

7. LITERATURE

1. Scott DL, Wolfe F, Huizinga TW: **Rheumatoid arthritis**. *Lancet (London, England)* 2010, **376**(9746):1094-1108.
2. Klareskog L, Catrina AI, Paget S: **Rheumatoid arthritis**. *Lancet (London, England)* 2009, **373**(9664):659-672.
3. Bax M, Huizinga TW, Toes RE: **The pathogenic potential of autoreactive antibodies in rheumatoid arthritis**. *Seminars in immunopathology* 2014, **36**(3):313-325.
4. Lefevre S, Meier FM, Neumann E, Muller-Ladner U: **Role of synovial fibroblasts in rheumatoid arthritis**. *Current pharmaceutical design* 2015, **21**(2):130-141.
5. Lefevre S, Knedla A, Tennie C, Kampmann A, Wunrau C, Dinser R, Korb A, Schnaker EM, Tarner IH, Robbins PD *et al*: **Synovial fibroblasts spread rheumatoid arthritis to unaffected joints**. *Nature medicine* 2009, **15**(12):1414-1420.
6. Karouzakis E, Gay RE, Michel BA, Gay S, Neidhart M: **DNA hypomethylation in rheumatoid arthritis synovial fibroblasts**. *Arthritis and rheumatism* 2009, **60**(12):3613-3622.
7. Karouzakis E, Gay RE, Gay S, Neidhart M: **Epigenetic control in rheumatoid arthritis synovial fibroblasts**. *Nature reviews Rheumatology* 2009, **5**(5):266-272.
8. Skeoch S, Bruce IN: **Atherosclerosis in rheumatoid arthritis: is it all about inflammation?** *Nature reviews Rheumatology* 2015, **11**(7):390-400.
9. Dalakas MC: **Inflammatory Muscle Diseases**. *The New England journal of medicine* 2015, **373**(4):393-394.
10. Vencovský J: **Idiopatické zánětlivé myopatie**. In: *Revmatologie.* Edited by Pavelka V, Horák, Šenolt, Mann, Štěpán. Praha: MAxdorf; 2012: 382-392.
11. Hill CL, Zhang Y, Sigurgeirsson B, Pukkala E, Mellemkjaer L, Airio A, Evans SR, Felson DT: **Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study**. *Lancet (London, England)* 2001, **357**(9250):96-100.
12. Zampieri S, Valente M, Adami N, Biral D, Ghirardello A, Rampudda ME, Vecchiato M, Sarzo G, Corbianco S, Kern H *et al*: **Polymyositis, dermatomyositis and malignancy: a further intriguing link**. *Autoimmunity reviews* 2010, **9**(6):449-453.
13. Ghirardello A, Borella E, Beggio M, Franceschini F, Fredi M, Doria A: **Myositis autoantibodies and clinical phenotypes**. *Auto- immunity highlights* 2014, **5**(3):69-75.
14. Tansley SL, McHugh NJ: **Myositis specific and associated autoantibodies in the diagnosis and management of juvenile and adult idiopathic inflammatory myopathies**. *Current rheumatology reports* 2014, **16**(12):464.
15. Suzuki R, Honda S, Kirino Y: **PIWI Expression and Function in Cancer**. *Frontiers in genetics* 2012, **3**:204.
16. Esteller M: **Non-coding RNAs in human disease**. *Nature reviews Genetics* 2011, **12**(12):861-874.
17. Filkova M, Jungel A, Gay RE, Gay S: **MicroRNAs in rheumatoid arthritis: potential role in diagnosis and therapy**. *BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy* 2012, **26**(3):131-141.
18. Ross RJ, Weiner MM, Lin H: **PIWI proteins and PIWI-interacting RNAs in the soma**. *Nature* 2014, **505**(7483):353-359.
19. Luteijn MJ, Ketting RF: **PIWI-interacting RNAs: from generation to transgenerational epigenetics**. *Nature reviews Genetics* 2013, **14**(8):523-534.
20. Rajasethupathy P, Antonov I, Sheridan R, Frey S, Sander C, Tuschl T, Kandel ER: **A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity**. *Cell* 2012, **149**(3):693-707.
21. Bresnick AR, Weber DJ, Zimmer DB: **S100 proteins in cancer**. *Nature reviews Cancer* 2015, **15**(2):96-109.
22. Pili R, Haggman M, Stadler WM, Gingrich JR, Assikis VJ, Bjork A, Nordle O, Forsberg G, Carducci MA, Armstrong AJ: **Phase II randomized, double-blind, placebo-controlled study of tasquinimod in men with minimally symptomatic metastatic castrate-resistant prostate cancer**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011, **29**(30):4022-4028.
23. Foell D, Kane D, Bresnihan B, Vogl T, Nacken W, Sorg C, Fitzgerald O, Roth J: **Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis**. *Rheumatology (Oxford, England)* 2003, **42**(11):1383-1389.
24. Youssef P, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, Bresnihan B: **Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane**. *The Journal of rheumatology* 1999, **26**(12):2523-2528.

25. Senolt L, Grigorian M, Lukanidin E, Simmen B, Michel BA, Pavelka K, Gay RE, Gay S, Neidhart M: **S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases**. *Annals of the rheumatic diseases* 2006, **65**(12):1645-1648.
26. Oslejskova L, Grigorian M, Hulejova H, Vencovsky J, Pavelka K, Klingelhofer J, Gay S, Neidhart M, Brabcova H, Suchy D *et al*: **Metastasis-inducing S100A4 protein is associated with the disease activity of rheumatoid arthritis**. *Rheumatology (Oxford, England)* 2009, **48**(12):1590-1594.
27. Hammer HB, Odegard S, Fagerhol MK, Landewe R, van der Heijde D, Uhlig T, Mowinckel P, Kvien TK: **Calprotectin (a major leucocyte protein) is strongly and independently correlated with joint inflammation and damage in rheumatoid arthritis**. *Annals of the rheumatic diseases* 2007, **66**(8):1093-1097.
28. Cerezo LA, Kuncova K, Mann H, Tomcik M, Zamecnik J, Lukanidin E, Neidhart M, Gay S, Grigorian M, Vencovsky J *et al*: **The metastasis promoting protein S100A4 is increased in idiopathic inflammatory myopathies**. *Rheumatology (Oxford, England)* 2011, **50**(10):1766-1772.
29. Fojtíková M: **Imunologické vyšetřvací metody v revmatologii**. In: *Revmatologie.* Edited by K Pavelka JV, P Horák, L Šenolt, H Mann, J Štěpán. Praha: Maxdorf; 2012: 40-57.
30. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD *et al*: **2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative**. *Annals of the rheumatic diseases* 2010, **69**(9):1580-1588.
31. Bohan A, Peter JB: **Polymyositis and dermatomyositis (first of two parts)**. *N Engl J Med* 1975, **292**(7):344-347.
32. Bohan A, Peter JB: **Polymyositis and dermatomyositis (second of two parts)**. *N Engl J Med* 1975, **292**(8):403-407.
33. Hoogendijk JE, Amato AA, Lecky BR, Choy EH, Lundberg IE, Rose MR, Vencovsky J, de Visser M, Hughes RA: **119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10-12 October 2003, Naarden, The Netherlands**. *Neuromuscul Disord* 2004, **14**(5):337-345.
34. Griggs RC, Askanas V, DiMauro S, Engel A, Karpati G, Mendell JR, Rowland LP: **Inclusion body myositis and myopathies**. *Ann Neurol* 1995, **38**(5):705-713.
35. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL: **Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis**. *Arthritis and rheumatism* 1995, **38**(1):44-48.
36. Scott DL, Houssien DA: **Joint assessment in rheumatoid arthritis**. *Br J Rheumatol* 1996, **35 Suppl 2**:14-18.
37. Isenberg DA, Allen E, Farewell V, Ehrenstein MR, Hanna MG, Lundberg IE, Oddis C, Pilkington C, Plotz P, Scott D *et al*: **International consensus outcome measures for patients with idiopathic inflammatory myopathies. Development and initial validation of myositis activity and damage indices in patients with adult onset disease**. *Rheumatology (Oxford, England)* 2004, **43**(1):49-54.
38. Betteridge Z, Gunawardena H, North J, Slinn J, McHugh N: **Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia**. *Rheumatology (Oxford, England)* 2007, **46**(6):1005-1008.
39. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS *et al*: **The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis**. *Arthritis and rheumatism* 1988, **31**(3):315-324.
40. Jungel A, Distler JH, Kurowska-Stolarska M, Seemayer CA, Seibl R, Forster A, Michel BA, Gay RE, Emmrich F, Gay S *et al*: **Expression of interleukin-21 receptor, but not interleukin-21, in synovial fibroblasts and synovial macrophages of patients with rheumatoid arthritis**. *Arthritis and rheumatism* 2004, **50**(5):1468-1476.
41. Sai Lakshmi S, Agrawal S: **piRNABank: a web resource on classified and clustered Piwi-interacting RNAs**. *Nucleic acids research* 2008, **36**(Database issue):D173-177.
42. Varet H, Coppée J-Y, Dillies M-A: **SARTools: a DESeq2- and edgeR-based R pipeline for comprehensive differential analysis of RNA-Seq data**; 2015.
43. Ke N, Wang X, Xu X, Abassi YA: **The xCELLigence system for real-time and label-free monitoring of cell viability**. *Methods Mol Biol* 2011, **740**:33-43.
44. Filkova M, Aradi B, Senolt L, Ospelt C, Vettori S, Mann H, Filer A, Raza K, Buckley CD, Snow M *et al*: **Association of circulating miR-223 and miR-16 with disease activity in patients with early rheumatoid arthritis**. *Annals of the rheumatic diseases* 2014, **73**(10):1898-1904.
45. Neumann E, Lefevre S, Zimmermann B, Gay S, Muller-Ladner U: **Rheumatoid arthritis progression mediated by activated synovial fibroblasts**. *Trends in molecular medicine* 2010, **16**(10):458-468.

46. Sugimoto K, Kage H, Aki N, Sano A, Kitagawa H, Nagase T, Yatomi Y, Ohishi N, Takai D: **The induction of H3K9 methylation by PIWIL4 at the p16Ink4a locus**. *Biochemical and biophysical research communications* 2007, **359**(3):497-502.
47. Cox DN, Chao A, Baker J, Chang L, Qiao D, Lin H: **A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal**. *Genes & development* 1998, **12**(23):3715-3727.
48. Mei Y, Clark D, Mao L: **Novel dimensions of piRNAs in cancer**. *Cancer letters* 2013, **336**(1):46-52.
49. Martinez VD, Vucic EA, Thu KL, Hubaux R, Enfield KS, Pikor LA, Becker-Santos DD, Brown CJ, Lam S, Lam WL: **Unique somatic and malignant expression patterns implicate PIWI-interacting RNAs in cancer-type specific biology**. *Scientific reports* 2015, **5**:10423.
50. Kang HJ, Moon MJ, Lee HY, Han SW: **Testosterone alters testis function through regulation of piRNA expression in rats**. *Molecular biology reports* 2014, **41**(10):6729-6735.
51. Pan Y, Hu M, Liang H, Wang JJ, Tang LJ: **The expression of the PIWI family members miwi and mili in mice testis is negatively affected by estrogen**. *Cell and tissue research* 2012, **350**(1):177-181.
52. M. Misunova GS-R, S. Luthin, C. Pommerenke, M. Fojtikova, J. Zavada, L. Plestilova, T. Svitalkova, P. Cepek, P. Novota, J. Vencovsky: **Microarray analysis of circulating micro RNAs in the serum of patients with polymyositis and dermatomyositis reveals a distinct disease expression profile and is associated with disease activity**. *Clinical and experimental rheumatology* 2015.
53. Carlsen AL, Schetter AJ, Nielsen CT, Lood C, Knudsen S, Voss A, Harris CC, Hellmark T, Segelmark M, Jacobsen S *et al*: **Circulating microRNA expression profiles associated with systemic lupus erythematosus**. *Arthritis and rheumatism* 2013, **65**(5):1324-1334.
54. Skoglund C, Carlsen AL, Weiner M, Kurz T, Hellmark T, Eriksson P, Heegaard NH, Segelmark M: **Circulating microRNA expression pattern separates patients with anti-neutrophil cytoplasmic antibody associated vasculitis from healthy controls**. *Clinical and experimental rheumatology* 2015, **33**(2 Suppl 89):S-64-71.
55. Oshikawa Y, Jinnin M, Makino T, Kajihara I, Makino K, Honda N, Nakayama W, Inoue K, Fukushima S, Ihn H: **Decreased miR-7 expression in the skin and sera of patients with dermatomyositis**. *Acta dermato-venereologica* 2013, **93**(3):273-276.
56. Shimada S, Jinnin M, Ogata A, Makino T, Kajihara I, Makino K, Honda N, Nakayama W, Inoue K, Fukushima S *et al*: **Serum miR-21 levels in patients with dermatomyositis**. *Clinical and experimental rheumatology* 2013, **31**(1):161-162.
57. Bao MH, Feng X, Zhang YW, Lou XY, Cheng Y, Zhou HH: **Let-7 in cardiovascular diseases, heart development and cardiovascular differentiation from stem cells**. *International journal of molecular sciences* 2013, **14**(11):23086-23102.
58. Lundberg IE: **Cardiac involvement in autoimmune myositis and mixed connective tissue disease**. *Lupus* 2005, **14**(9):708-712.
59. Plestilova L, Mann H, Andres Cerezo L, Pecha O, Vencovsky J, Senolt L: **The metastasis promoting protein S100A4 levels associate with disease activity rather than cancer development in patients with idiopathic inflammatory myopathies**. *Arthritis research & therapy* 2014, **16**(5):468.
60. Helfman DM, Kim EJ, Lukanidin E, Grigorian M: **The metastasis associated protein S100A4: role in tumour progression and metastasis**. *British journal of cancer* 2005, **92**(11):1955-1958.
61. Klingelhofer J, Grum-Schwensen B, Beck MK, Knudsen RS, Grigorian M, Lukanidin E, Ambartsumian N: **Anti-S100A4 antibody suppresses metastasis formation by blocking stroma cell invasion**. *Neoplasia* 2012, **14**(12):1260-1268.
62. Wang L, Wang X, Liang Y, Diao X, Chen Q: **S100A4 promotes invasion and angiogenesis in breast cancer MDA-MB-231 cells by upregulating matrix metalloproteinase-13**. *Acta Biochim Pol* 2012, **59**(4):593-598.
63. Zahr ZA, Baer AN: **Malignancy in myositis**. *Curr Rheumatol Rep* 2011, **13**(3):208-215.
64. Klingelhofer J, Senolt L, Baslund B, Nielsen GH, Skibshoj I, Pavelka K, Neidhart M, Gay S, Ambartsumian N, Hansen BS *et al*: **Up-regulation of metastasis-promoting S100A4 (Mts-1) in rheumatoid arthritis: putative involvement in the pathogenesis of rheumatoid arthritis**. *Arthritis Rheum* 2007, **56**(3):779-789.
65. Novitskaya V, Grigorian M, Kriajevska M, Tarabykina S, Bronstein I, Berezin V, Bock E, Lukanidin E: **Oligomeric forms of the metastasis-related Mts1 (S100A4) protein stimulate neuronal differentiation in cultures of rat hippocampal neurons**. *J Biol Chem* 2000, **275**(52):41278-41286.
66. Cerezo LA, Kuncová K, Mann H, Tomcík M, Zámecník J, Lukanidin E, Neidhart M, Gay S, Grigorian M, Vencovsky J *et al*: **The metastasis promoting protein S100A4 is increased in idiopathic inflammatory myopathies**. *Rheumatology (Oxford)* 2011, **50**(10):1766-1772.
67. Cerezo LA, Remakova M, Tomcik M, Gay S, Neidhart M, Lukanidin E, Pavelka K, Grigorian M, Vencovsky J, Senolt L: **The metastasis-associated protein S100A4 promotes the inflammatory**

response of mononuclear cells via the TLR4 signalling pathway in rheumatoid arthritis. *Rheumatology (Oxford)* 2014.
68. Bianchi ME: **DAMPs, PAMPs and alarmins: all we need to know about danger**. *Journal of leukocyte biology* 2007, **81**(1):1-5.
69. Andres Cerezo L, Mann H, Pecha O, Plestilova L, Pavelka K, Vencovsky J, Senolt L: **Decreases in serum levels of S100A8/9 (calprotectin) correlate with improvements in total swollen joint count in patients with recent-onset rheumatoid arthritis**. *Arthritis research & therapy* 2011, **13**(4):R122.
70. Brun JG, Haga HJ, Boe E, Kallay I, Lekven C, Berntzen HB, Fagerhol MK: **Calprotectin in patients with rheumatoid arthritis: relation to clinical and laboratory variables of disease activity**. *The Journal of rheumatology* 1992, **19**(6):859-862.
71. Sunahori K, Yamamura M, Yamana J, Takasugi K, Kawashima M, Yamamoto H, Chazin WJ, Nakatani Y, Yui S, Makino H: **The S100A8/A9 heterodimer amplifies proinflammatory cytokine production by macrophages via activation of nuclear factor kappa B and p38 mitogen-activated protein kinase in rheumatoid arthritis**. *Arthritis research & therapy* 2006, **8**(3):R69.
72. Garcia-De La Torre I: **Clinical Usefulness of Autoantibodies in Idiopathic Inflammatory Myositis**. *Frontiers in immunology* 2015, **6**:331.
73. Klein M, Mann H, Plestilova L, Betteridge Z, McHugh N, Remakova M, Novota P, Vencovsky J: **Arthritis in idiopathic inflammatory myopathy: clinical features and autoantibody associations**. *The Journal of rheumatology* 2014, **41**(6):1133-1139.
74. Oddis CV, Medsger TA, Jr., Cooperstein LA: **A subluxing arthropathy associated with the anti-Jo-1 antibody in polymyositis/dermatomyositis**. *Arthritis and rheumatism* 1990, **33**(11):1640-1645.
75. Mielnik P, Wiesik-Szewczyk E, Olesinska M, Chwalinska-Sadowska H, Zabek J: **Clinical features and prognosis of patients with idiopathic inflammatory myopathies and anti-Jo-1 antibodies**. *Autoimmunity* 2006, **39**(3):243-247.
76. Mumm GE, McKown KM, Bell CL: **Antisynthetase syndrome presenting as rheumatoid-like polyarthritis**. *Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases* 2010, **16**(7):307-312.
77. Nakajima A, Yoshino K, Soejima M, Kawaguchi Y, Satoh T, Kuwana M, Yamanaka H: **High frequencies and co-existing of myositis-specific autoantibodies in patients with idiopathic inflammatory myopathies overlapped to rheumatoid arthritis**. *Rheumatology international* 2012, **32**(7):2057-2061.
78. Klein M, Mann H, Plestilova L, Zamecnik J, Betteridge Z, McHugh N, Vencovsky J: **Increasing incidence of immune-mediated necrotizing myopathy: single-centre experience**. *Rheumatology (Oxford, England)* 2015.
79. Mammen AL, Chung T, Christopher-Stine L, Rosen P, Rosen A, Doering KR, Casciola-Rosen LA: **Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy**. *Arthritis and rheumatism* 2011, **63**(3):713-721.
80. Osaki Y, Nakagawa Y, Miyahara S, Iwasaki H, Ishii A, Matsuzaka T, Kobayashi K, Yatoh S, Takahashi A, Yahagi N *et al*: **Skeletal muscle-specific HMG-CoA reductase knockout mice exhibit rhabdomyolysis: A model for statin-induced myopathy**. *Biochemical and biophysical research communications* 2015.
81. Robinson JG: **Starting primary prevention earlier with statins**. *The American journal of cardiology* 2014, **114**(9):1437-1442.

8. LIST OF PUBLICATIONS

Publications on the PhD. thesis topic with IF:

Pleštilová L, Neidhart M, Russo G, Frank-Bertoncelj M, Ospelt C, Ciurea A, Kolling C, Gay RE, Michel BA, Vencovský J, Gay S, Jüngel A. PIWI proteins and PIWI-interacting RNAs in rheumatoid arthritis Manuscript submitted to Annals of Rheumatic Diseases (IF 10,377)

Misunova M, Salinas-Riester G, Luthin S, Pommerenke C, Fojtikova M, Zavada J, Klein M, **Plestilova L**, Svitalkova T, Cepek P, Novota P, Vencovsky J. Microarray analysis of circulating micro RNAs in the serum of patients with polymyositis and dermatomyositis reveals a distinct disease expression profile and is associated with disease activity, Clin Exp. Rheumatology, Manuscript Number: CER 8302 15 IF 2,9

Pleštilová L, Mann H, Andrés Cerezo L, Pecha O, Vencovský J, Senolt L. The metastasis promoting protein S100A4 levels associate with disease activity rather than cancer development in patients with idiopathic inflammatory myopathies. Arthritis Res Ther. 2014 Oct 31;16(5):468. IF 4,12

Andrés Cerezo L, Mann H, Pecha O, **Pleštilová L**, Pavelka K, Vencovský J, Senolt L. Decreases in serum levels of S100A8/9 (calprotectin) correlate with improvements in total swollen joint count in patients with recent-onset rheumatoid arthritis. Arthritis Res Ther. 2011 Jul 26;13(4):R122. IF 4,45

Klein M, Mann H, **Pleštilová L**, Betteridge Z, McHugh N, Remáková M, Novota P, Vencovský J. Arthritis in idiopathic inflammatory myopathy: clinical features and autoantibody associations. J Rheumatol. 2014 Jun;41(6):1133-9. IF 3,2

Klein M, Mann H, **Pleštilová L**, Zámečník J, Betteridge Z, McHugh N, Vencovský J. Increasing incidence of immune-mediated necrotizing myopathy: single-centre experience. Rheumatology (Oxford). 2015 Jun 24. pii: kev229. IF 4,47

Publications on the PhD. thesis topic reviewed:

Pleštilová L, Vencovský J. Anti-PM-Scl autoprotilátky u myozitid. Čes. Revmatol. 2009; 17(4): 200-204.

Other publications with IF:

Herbert MK, Stammen-Vogelzangs J, Verbeek MM, Rietveld A, Lundberg IE, Chinoy H, Lamb JA, Cooper RG, Roberts M, Badrising UA, De Bleecker JL, Machado PM, Hanna MG, **Plestilova L**, Vencovsky J, van Engelen BG, Pruijn GJ. Disease specificity of autoantibodies to cytosolic 5'-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. Ann Rheum Dis. 2015 Feb 24. pii: annrheumdis-2014-206691. doi: 10.1136/annrheumdis-2014-206691. IF 9,27

Šenolt L, Cerezo LA, Šumová B, Pecha O, **Pleštilová L**, Forejtová Š, Růžičková O, Hušáková M, Závada J, Pavelka K, Vencovský J, Mann H. High levels of metastasis-inducing S100A4 protein and treatment outcome in early rheumatoid arthritis: data from the PERAC cohort. Biomarkers. 2015 Feb;20(1):47-51. IF 2.2

Sglunda O, Mann H, Hulejová H, Kuklová M, Pecha O, **Pleštilová L**, Filková M, Pavelka K, Vencovský J, Senolt L. Decreased Circulating Visfatin Is Associated with Improved Disease Activity in Early Rheumatoid Arthritis: Data from the PERAC Cohort. PLoS One. 2014 Jul 28;9(7):e103495. IF 3,5

Sglunda O, Mann HF, Hulejová H, Pecha O, **Pleštilová L**, Růžičková O, Fojtíková M, Sléglová O, Forejtová S, Pavelka K, Vencovský J, Senolt L. Decrease in serum interleukin 21 levels is associated with disease activity improvement in patients with recent-onset rheumatoid arthritis. Physiol Res. 2014 Apr 3. IF 1,5

Filková M, Hulejová H, Kuncová K, **Pleštilová L**, Cerezo LA, Mann H, Klein M, Zámečník J, Gay S, Vencovský J, Senolt L. Resistin in idiopathic inflammatory myopathies. Arthritis Res Ther. 2012 May 11;14(3):R111. IF 4,45

9. LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ANA	antinuclear antibodies
anti-CCP	anti-cyclic citrullinated peptide antibody
anti-HMGCR	anti-hydroxy-methyl-glutaryl-coenzym A reductasis antibody
anti-SRP	anti-signal recognition particle antibody
anti-TIF1-γ	anti-transcriptional intermediary factor 1γ antibody
CAM	cancer associated myositis
CK	creatine kinase
CRP	C-reaktivní protein
DAS28	disease activity score of 28 joints
DAS28	disease activity
DM	dermatomyozitida
DMARD	disease modifying antirheumatic drugs
GC	glucocorticoids
HAQ	health assessment questionnaire
HC	healthy controls
HDAC	histone deacetylase
HLA	human leukocytar antigen
IBM	inclusion body myositis
IIM	idiopathic inflammatory myositis
IL	interleukine
IMNM	immune mediated necrotizing myopathy
IZM	idiopatické zánětlivé myopatie
LPS	lipopolysaccharide
MMT	manual muscle test
mRNA	messenger RNA
MYOACT	myositis disease activity assessments visual analog scales
OA	osteoartróza
piRNA	PIWI-interagující RNA
PIWI	P-element Induced Wimpy testis
PIWIL	PIWI-like
PM	polymyozitida
RA	revmatoidní artritida
RF	revmatoidní faktor

SD	standart deviation
SEM	standart error of the mean
SF	synoviální fibroblasty
SJC	swollen joint count
SLE	systémový lupus erythematodes
TLR	toll-like receptor
TNF-α	tumor necrosis factor α
VAS	visual analogue scale

ACKNOWLEDGEMENTS

I would like to thank to my supervisor Prof. Jiří Vencovský from the Institute of Rheumatology in Prague for excellent guidance, advice and discussions. I also thank Prof. Ladislav Šenolt, it was great to work with you and I am very grateful for the opportunity you gave me to visit the Center of experimental rheumatology in Zurich. Big thanks to the directors of both institutions Prof. Karel Pavelka (Prague) and Prof. Steffan Gay and Prof. Renate Gay (Zurich) for making this possible. Many thanks to Dr. Astrid Jüngel, PD and Prof. Michel Neidhart in Zurich, who guided me carefully through the world of epigenetics.

Warmest thanks to my colleagues and friends from both groups. Marek, Olinka, Martin, Lucka, Martina, Markéta, Mária, Michal, Mojca, Anna, Borbola, Fang Fang, Clare, Emmanuel, Caroline, Kerstin, Peter and Bea, it was a great pleasure to work with you and to learn from you.

The final thanks are to my family. Mamulko, taťko, Andulko moc moc moc děkuju za všechno a mám vás moc ráda. Guigui, merci pour tout et je t'aime.

Lenka