The arthritis severity locus Cia5a regulates the expression of inflammatory mediators including Syk pathway genes and proteases in pristane-induced arthritis

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Abstract

Background: Cia5a is a locus on rat chromosome 10 that regulates disease severity and joint damage in two models of rheumatoid arthritis, collagen- and pristane-induced arthritis (PIA). In this study, we aimed to identify cellular and molecular processes regulated by Cia5a using microarray-based gene expression analysis of synovial tissues from MHC identical DA (severe erosive disease) and DA.F344(Cia5a) congenics (mild non-erosive disease) rats.

Results: Synovial tissues from six DA and eight DA.F344(Cia5a) rats were analyzed 21 days after the induction of PIA using the Illumina RatRef-12 BeadChip (21,922 genes) and selected data confirmed with qPCR. There was a significantly increased expression of pro-inflammatory mediators such as Il1b (5-fold), Il18 (3.9-fold), Cxcl1 (10-fold), Cxcl13 (7.5-fold) and Ccl7 (7.9-fold), and proteases like Mmp3 (23-fold), Mmp9 (32-fold), Mmp14 (4.4-fold) and cathepsins in synovial tissues from DA, with reciprocally reduced levels in congenics. mRNA levels of 47 members of the Spleen Tyrosine Kinase (Syk) pathway were significantly increased in DA synovial tissues compared with DA.F344(Cia5a), and included Syk (5.4-fold), Syk-activating receptors and interacting proteins, and genes regulated by Syk such as NFkB, and NAPDH oxidase complex genes. Nuclear receptors (NR) such as Rxrg, Pparg and Rev-erba were increased in the protected congenics, and so was the anti-inflammatory NR-target gene Scd1 (54-fold increase). Tnn (72-fold decrease) was the gene most significantly increased in DA.

Conclusions: Analyses of gene expression in synovial tissues revealed that the arthritis severity locus Cia5a regulates the expression of key mediators of inflammation and joint damage, as well as the expression of members of the Syk pathway. This expression pattern correlates with disease severity and joint damage and along with the gene accounting for Cia5a could become a useful biomarker to identify patients at increased risk for severe and erosive disease. The identification of the gene accounting for Cia5a has the potential to generate a new and important target for therapy and prognosis.

Keywords: Rheumatoid arthritis, Articular damage, Autoimmune
Background

Rheumatoid arthritis (RA) is a common, chronic and potentially debilitating form of autoimmune erosive arthritis. Advances in the understanding of RA pathogenesis have led to the development of new and better treatments [1-3]. Yet, sustained remission is still rarely achieved [4], and more effective therapies are needed.

The identification of genes implicated in the regulation of arthritis severity and articular damage has the potential to generate new and potentially better targets for therapies aimed at preserving joint architecture and function, and reducing the risk of developing joint deformities. Yet, little is known about those genes [5], and the large cohorts of RA patients used in genome-wide association studies for susceptibility were not designed to address disease severity and articular damage.

We have previously identified several disease severity and articular damage quantitative trait loci (QTL) in rat models of RA [6-10]. Using a combination of positional cloning and functional studies that include transcriptome analyses of synovial cells and synovial tissues we are beginning to understand the molecular processes regulating arthritis severity and joint damage in pristane- and collagen-induced arthritis (PIA and CIA) [10-14]. Similar analyses have been successfully used to identify other autoimmunity genes in rodent models [15,16].

Cia5a is a 20.6Mb QTL on rat chromosome 10 that regulates arthritis severity, cartilage and bone damage, synovial hyperplasia and inflammation in both PIA and CIA [9,10]. In the present study we used synovial tissues from arthritis-protected DA.F344(Cia5a) congenics and from arthritis-susceptible and MHC-identical DA rats in a microarray analysis of gene expression. We determined that the Cia5a locus regulates the expression of several genes central to RA pathogenesis and joint damage, such as cytokines Il1b and Il18, chemokines, proteases, mediators of the synthesis of reactive oxygen species and prostaglandins, and genes involved in Toll-like receptor signaling. Additionally, the expression of 47 members of the Syk kinase pathway genes, including NfkB genes were significantly regulated by the Cia5a locus. Furthermore, the presence of F344 alleles at the Cia5a interval was associated with increased expression of anti-inflammatory genes, including nuclear receptors and Timp3, suggesting that the Cia5a locus contains a gene involved in maintaining an inflammation-free synovial tissue.

Results

DA.F344(Cia5a) congenics develop a mild form of PIA with a distinct pattern of gene expression compared with DA rats

DA.F344(Cia5a) rats developed a significantly milder form of PIA compared with DA rats (median arthritis severity score (25–75 percentiles), DA=26.5 (17–36.9), DA.F344(Cia5a)=5.5 (3.6–7.2); p=0.002, Mann–Whitney test; Figure 1A and B).

36% (7,925) of the genes in the RatRef-12 BeadChip were consistently expressed in synovial tissues. Nearly one-third of these genes (2,648) met the filtering criteria for differential expression (fold-difference ≥1.5 and p≤0.01). The presence of F344 alleles at the Cia5a interval, as in DA.F344(Cia5a) congenic rats, was associated with increased expression of 1,241 genes and reduced expression of 1,407 genes compared with DA. 134 genes had a ≥5-fold difference between strains (Figure 1C). 46 genes had a ≥10-fold difference in expression, of which 19 were increased and 27 decreased in congenics, compared with DA (Tables 1 and 2).

Expression of pro-inflammatory genes, proteases (including matrix metalloproteases, MMPs) and adhesion molecules was significantly increased in DA and decreased in DA.F344(Cia5a)

The 1,407 genes with increased expression in DA and reciprocally decreased expression in DA.F344(Cia5a) congenics included pro-inflammatory cytokines and chemokines implicated in arthritis pathogenesis such as Il1b (5.17-fold on microarray, and 2.46-fold on qPCR), Il18, Mif, Ccl2, Ccl7 and Cxcl13 (Table 3 and Additional file 1: Table S3 and Additional file 2: Table S4). Genes with significantly decreased expression in congenics also included those implicated in the development of cartilage and bone erosions such as MMPs (Mmp3 [24-fold], Mmp9 and Mmp14), and other proteases (cathepsins D, E, K and S) (Table 3 and Figure 2). Interestingly, Syk (see below) has been shown to regulate the expression of differentially expressed MMPs such as Mmp3 [17] and Mmp9 [18], further suggesting a potential central role for Syk in arthritis and a Syk-regulatory effect of Cia5a. Components of the extracellular matrix (ECM; Cthrc1, Col12a1, Emilin1) also had reduced expression in congenics, and together with the levels of proteases suggested that there was reduced matrix turnover and reduced degradation, compared with arthritic DA rats (Table 3).

Adhesion molecules required for leukocyte migration into the synovium were increased in DA synovial tissues and decreased in DA.F344(Cia5a), including integrins Itga5, Itgam, Itgb2, Itgb7, and Cd44 (Table 3). Cadherin-11 (Cd11), a FLS-specific gene required for cell-cell interactions and implicated in FLS invasion and synovial hyperplasia was also decreased in DA.F344(Cia5a) congenics, consistent with the non-hyperplastic synovial tissue previously described in this strain, as opposed to the highly hyperplastic synovial tissue seen in DA [10].

The gene with the most significantly increased expression in DA versus DA.F344(Cia5a) was Tnn (Tenascin
Tnn has been implicated in osteogenesis and angiogenesis but not in arthritis or inflammation. These results demonstrate that DA rats with PIA have increased synovial expression of many genes implicated in RA pathogenesis, further validating the molecular similarities between PIA and RA, and underscoring the potential relevance of both Cia5a in arthritis pathogenesis and the present study in discovering new key genes and pathways regulating arthritis.

**Figure 1** DA and DA.F344(Cia5a) rats differ in arthritis severity and have different synovial gene expression profiles. (A) Map of the Cia5a locus on rat chromosome 10, and the congenic interval boundaries (black=homozygous for F344 alleles; white=homozygous for DA alleles; grey=recombination interval). (B) DA rats had severe disease at 21 days post-induction of PIA; DA.F344(Cia5a) congenics were protected and developed a significantly milder form of arthritis (p=0.002, Mann–Whitney test; boxes show the median and 25%-75% percentiles). (C) 7,925 genes were expressed in all synovial tissues. 2,648 (33.4%) met the 1.5-fold difference and p-value of ≤0.01 (t-test) for significant difference. 134 genes differed by ≥5-fold and less <10-fold, and 46 genes differed by ≥10-fold (inset).

**Table 1** Genes with ≥10-fold reduction in expression in DA.F344(Cia5a) compared with DA

| Symbol | Name                                      | Entrez Gene ID | Fold reduction | p-value  |
|--------|-------------------------------------------|----------------|----------------|----------|
| Tnn    | Tenascin N                                | 304913         | 71.69          | 8.6x10^{-11} |
| Mmp9   | Matrix metallopeptidase 9                 | 81687          | 32.79          | 1.4x10^{-9}  |
| Cdc2   | Cell division cycle 2, G1 to S and G2 to M| 54237          | 24.16          | 1.6x10^{-5}  |
| Mmp3   | Matrix metallopeptidase 3                 | 171045         | 23.94          | 5.0x10^{-5}  |
| Ccnb2  | Cyclin B2                                 | 363088         | 22.95          | 5.0x10^{-6}  |
| Chthc1 | Collagen triple helix repeat containing 1  | 282836         | 17.93          | 3.2x10^{-9}  |
| Col12a1| Collagen, type XII, alpha 1               | 25683          | 16.25          | 7.6x10^{-8}  |
| Slpi   | Secretory leukocyte peptidase inhibitor   | 84386          | 16.16          | 6.4x10^{-4}  |
| Spc24  | SPC24, NDC80 kinetochore complex component, homolog | 363028 | 14.72 | 2.5x10^{-6} |
| Emilin1| Elastin microfibril interactor 1          | 298845         | 13.55          | 2.4x10^{-6}  |
| Prc1   | Protein regulator of cytokinesis 1        | 308761         | 13.08          | 1.6x10^{-5}  |
| Embg   | Embigin                                   | 114511         | 12.82          | 1.4x10^{-5}  |
| Nut2   | NUF2, NDC80 kinetochore complex component, homolog | 304951 | 12.52 | 2.5x10^{-6} |
| Lbp    | Lipopolysaccharide binding protein        | 29469          | 11.82          | 8.0x10^{-5}  |
| Cks2   | CDC28 protein kinase regulatory subunit 2 | 498709         | 11.62          | 5.9x10^{-6}  |
| Wisp1  | WNT1 inducible signaling pathway protein 1| 65154          | 11.59          | 9.0x10^{-8}  |
| Cxcl1  | Chemokine (C-x-C motif) ligand 1          | 81503          | 10.88          | 4.3x10^{-4}  |
| Steap1 | Six transmembrane epithelial antigen of the prostate 1 | 297738 | 10.61 | 1.7x10^{-4} |
| LOC687334| Similar to cytoskeleton associated protein 2 | 687334 | 10.16 | 9.1x10^{-6} |
Increased expression of members of the Syk (spleen tyrosine kinase) pathway in DA synovial tissues, and reciprocally decreased expression in DA.F344(Cia5a)

47 members of the Syk pathway were expressed in significantly increased levels in DA, and in reduced levels in congenics (Table 4, and Figures 2 and 3). These included: a) Syk-activating receptors such as FcgR2a, FcεR1g, integrins (Itgα5, Itgβ2, Itgαm), c-lectin receptors (Clec4A3, Clec7α [Dectin 1], Clec11Aα), Trem2 and Dap12 (Tyrobp), b) Syk (5.4-fold) itself, c) Syk-interacting and downstream signaling genes including Vav1, Lcp2 (Slp76), Ptk2b (Pyk2), Lat, Rac2, and Ezr (Vil2), and d) genes belonging to pathways activated by Syk and implicated in arthritis pathogenesis and synovial hyperplasia and pannus formation such as NfkB pathway genes (Fadd, Ikbkb, Nfkβ1, Nfkβ2), cytokines (Il1b, Ltb, Mif), genes implicated in cell proliferation (Ccnb2, Cdc2, Cks2, Nuf2), cytoskeleton regulation (Actr3, Arpc4, Coro1b, Ezr/Vil2, Myo9b, Parva, Tubb5), and NADPH oxidase complex genes implicated in the production of reactive oxygen species (ROS) (Ncf1, Ncf2, Ncf4, Cyba) (Table 4).

Interestingly, genes that neutralize ROS (Cat, Sod1, Gss) went on the opposite direction with increased expression in congenics (Table 5).

Additionally, Syk and Vav1 expression levels correlated with the cumulative arthritis severity score (Pearson’s correlation coefficient of 0.8 and p=0.0006 for both genes). Taken together, these observations suggest that the Cia5a QTL contains an arthritis gene that directly or indirectly regulates the expression of Syk pathway genes, providing a possible mechanistic explanation for this locus’ effect on the regulation of disease severity.

Table 2 Genes with ≥10-fold increased expression in DA.F344(Cia5a) compared with DA *

| Symbol | Name                                      | Entrez Gene ID | Fold increase | p-value       |
|--------|-------------------------------------------|----------------|---------------|---------------|
| Scd1   | Stearoyl-Coenzyme A desaturase 1           | 246074         | 54.62         | 8.8x10⁻⁵      |
| Mpz    | Myelin protein zero                       | 24564          | 39.80         | 7.7x10⁻⁵      |
| Akr1c19| Aldo-keto reductase family 1, member C19  | 307096         | 26.45         | 3.0x10⁻⁴      |
| Nnat   | Neuronatin                                | 94270          | 22.08         | 5.2x10⁻⁷      |
| Ces3   | Carboxylesterase 3                        | 113902         | 21.06         | 5.4x10⁻⁴      |
| Mup5   | Major urinary protein 5                   | 298107         | 18.41         | 9.5x10⁻⁵      |
| LOC688457 | Similar to Major urinary protein precursor (MUP) | 688457        | 17.42         | 1.7x10⁻⁴      |
| Abcd2  | ATP-binding cassette, sub-family D (ALD), member 2 | 84356       | 16.95         | 4.2x10⁻⁴      |
| S100b  | S100 calcium binding protein B             | 25742          | 16.81         | 8.9x10⁻⁵      |
| Tshr   | Thyroid stimulating hormone receptor      | 25360          | 16.61         | 1.8x10⁻⁷      |
| LOC689147 | Hypothetical protein LOC689147              | 689147         | 15.84         | 7.9x10⁻⁶      |
| Thrp   | Thyroid hormone responsive                | 25357          | 15.76         | 1.2x10⁻⁵      |
| LOC259244 | Alpha-2u globulin PGCL3                     | 259244         | 15.74         | 1.8x10⁻⁴      |
| Mup4   | Major urinary protein 4                   | 362527         | 15.38         | 9.9x10⁻⁵      |
| Omd    | Osteomodulin                              | 83717          | 13.82         | 5.8x10⁻⁴      |
| Plek hb1 | Pleckstrin homology domain containing, family B (evektins) member 1 | 64471       | 13.26         | 2.9x10⁻⁵      |
| Ankrd5 | Ankyn repeat domain 5                     | 296184         | 13.14         | 4.3x10⁻⁵      |
| Cidea  | Cell death-inducing DNA fragmentation factor,a subunit-like effector A | 291541      | 12.91         | 2.4x10⁻⁵      |
| Atp1a2 | ATPase, Na+/K+ transporting, alpha 2 polypeptide | 24212      | 12.62         | 6.9x10⁻⁵      |
| Pck1   | Phosphoenolpyruvate carboxykinase 1 (soluble) | 362282      | 11.95         | 2.4x10⁻⁵      |
| Mtap   | Melanocortin 2 receptor accessory protein | 288271         | 11.80         | 1.8x10⁻⁷      |
| Timp3  | TIMP metallopeptidase inhibitor 3          | 25358          | 11.48         | 2.9x10⁻⁴      |
| MGCT2973 | Beta-glo                                    | 361619         | 11.38         | 1.4x10⁻⁶      |
| Plp1   | Proteolipid protein 1                     | 24943          | 11.33         | 1.3x10⁻⁵      |
| Plin   | Perilin                                   | 25629          | 10.99         | 9.1x10⁻⁷      |
| Adipoq | Adiponectin, C1Q and collagen domain containing | 246253       | 10.96         | 3.3x10⁻⁶      |
| Pcd1   | Pterin-4 a-carboxaldehyde dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 a | 297080      | 10.74         | 5.8x10⁻⁸      |

* Bold = nuclear receptor-inducible gene.
Table 3 Mediators of inflammation and articular damage up-regulated in DA synovium and down-regulation in DA.F344(Cia5a)

| Gene Symbol | Gene Name                              | Entrez Gene ID | Fold DA/Cia5a | p-value*       |
|-------------|----------------------------------------|----------------|---------------|---------------|
| **Cytokines and chemokines**                      |                          |                |               |               |
| Il1b        | interleukin 1 beta                     | 24494          | 5.17          | 0.002         |
| Il18        | interleukin 18                         | 29197          | 3.91          | 0.0002        |
| Ltb         | lymphotoxin beta                       | 361795         | 3.77          | 0.0002        |
| Mif         | macrophage migration inhibitory factor | 81683          | 2.37          | 0.0002        |
| Aif1        | allograft inflammatory factor 1        | 29427          | 2.48          | 0.0001        |
| Ccl2        | chemokine (C-C motif) ligand 2         | 24770          | 3.95          | 0.01          |
| Ccl7        | chemokine (C-C motif) ligand 7         | 287561         | 7.90          | 0.002         |
| Cxcl1       | chemokine (C-X-C motif) ligand 1       | 81503          | 10.88         | 0.0004        |
| Cxcl13      | chemokine (C-X-C motif) ligand 13      | 498335         | 7.53          | 0.000004      |
| **Proteases**                                     |                          |                |               |               |
| Mmp3        | matrix metallopeptidase 3              | 171045         | 23.94         | 0.0001        |
| Mmp9        | matrix metallopeptidase 9              | 81687          | 32.79         | 0.000000001   |
| Mmp14       | matrix metallopeptidase 14             | 81707          | 4.46          | 0.00000004    |
| Mmp19       | matrix metallopeptidase 19             | 304608         | 5.63          | 0.0000003     |
| Ctsc        | cathepsin C                            | 25423          | 2.64          | 0.00007       |
| Ctsd        | cathepsin D                            | 171293         | 1.90          | 0.000002      |
| Ctse        | cathepsin E                            | 25424          | 2.03          | 0.0002        |
| Ctsk        | cathepsin K                            | 29175          | 3.20          | 0.000003      |
| Ctsl        | cathepsin S                            | 50654          | 1.68          | 0.002         |
| **Extra-cellular matrix**                         |                          |                |               |               |
| Cthrc1      | collagen triple helix repeat containing 1 | 282836    | 17.93         | 0.000000003   |
| Col12a1     | collagen, type XII, alpha 1            | 25683          | 16.25         | 0.00000008    |
| Emilin1     | elastin microfibril interfacier 1      | 298845         | 13.55         | 0.000002      |
| **Adhesion molecules**                            |                          |                |               |               |
| Itga5       | integrin alpha 5 (fibronectin receptor alpha) | 315346     | 3.47          | 0.00000001    |
| Itgam       | integrin alpha M                       | 25021          | 3.58          | 0.0001        |
| Itgav       | integrin alpha V                       | 296456         | 1.61          | 0.0004        |
| Itgb2       | integrin beta 2                        | 309684         | 3.44          | 0.0001        |
| Itgb7       | integrin, beta 7                       | 25713          | 3.86          | 0.002         |
| Cd44        | Cd44 molecule                          | 25406          | 1.83          | 0.005         |
| Cdh11       | cadherin 11                            | 84407          | 1.87          | 0.003         |
| **Toll-like receptors and regulators of their activity** |                          |                |               |               |
| Cd14        | CD14                                   | 60350          | 2.13          | 0.001         |
| Ira4        | interleukin-1 receptor-associated kinase 4 | 300177    | 1.65          | 0.0004        |
| Lptb        | lipopolysaccharide binding protein      | 29469          | 4.36          | 0.0003        |
| Myd88       | myeloid differentiation primary response gene 88 | 301059    | 1.59          | 0.002         |
| Pycard      | PYD and CARD domain containing         | 282817         | 1.95          | 0.0004        |
| Tlr2        | toll-like receptor 2                   | 310553         | 4.36          | 0.00          |
| Tlr6        | toll-like receptor 6                   | 305353         | 1.76          | 0.001         |
DA.F344(Cia5a) congenics have reduced synovial expression of innate immune response-activating genes, including members of the inflammasome

Expression levels of genes implicated in innate immune responses were significantly increased in DA, and decreased in DA.F344(Cia5a). In addition to the Syk pathway, and mediators of ROS synthesis, and regulators of cytokine transcription such as members of the NFκB pathways discussed above, DA.F344(Cia5a) congenics also had reduced expression of AP-1 genes (Fos and JunB), Il1b and other members of the inflammasome (Card11, Nalp3 [both detected only in DA], and Pycard). Pattern recognition receptors such as Zbp1 and Lgp2 (both detected only in DA), and components of the toll-like receptor (TLR) pathway (Cd14, Ikbke, Ira4, Lbp, MyD88, Tlr2, Tlr6, Ticam1; Table 3) were expressed in increased levels in DA and decreased in congenics. Interestingly, and in line with these observations, the expression levels of negative regulators of TLR signaling such as Ptpn11 and Pparg was conversely increased in congenics, suggesting that the arthritis gene located within the Cia5a QTL might mediate the balance between activating and inhibitory signals implicated in TLR signaling.

Genes implicated in the synthesis of prostaglandins and leukotrienes (Pla2g4a, Ptgs2/Cox2, Ptges) were also increased in DA (Table 3), while genes that counteract eicosanoid-mediated inflammation (Ptgis, Cyp2j3) were increased in congenics (Table 5).

Increased expression of anti-inflammatory genes, including nuclear receptors (NRS), in synovial tissues from DA.F344(Cia5a) Congenics

Several genes with known anti-inflammatory and cytokine-suppressing properties were expressed in increased levels in DA.F344(Cia5a) synovial tissues, and reduced in DA. These...
Table 4 Members of the Syk kinase pathway up-regulated in DA synovium compared with down-regulation in DA.F344(Cia5a)

| Gene Symbol | Gene Name                                                                 | Entrez Gene ID | Fold DA/Cia5a | p-value*   |
|-------------|---------------------------------------------------------------------------|----------------|---------------|------------|
| **Activating receptors** |                                                                       |                |               |            |
| Fcer1g      | Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide     | 25441          | 2.50          | 0.0004     |
| Fcgr2a      | Fc fragment of IgG, low affinity IIa, receptor (CD32)                     | 116591         | 3.08          | 0.005      |
| Tcrg        | T cell receptor gamma locus                                               | 24821          | 3.32          | 0.0001     |
| Trem2       | triggering receptor expressed on myeloid cells 2                         | 301227         | 2.69          | 0.0006     |
| Tyrobp      | Tyro protein tyrosine kinase binding protein                              | 361537         | 4.80          | 0.0001     |
| **Integrins** |                                                                       |                |               |            |
| Itgam       | integrin alpha M                                                          | 25021          | 3.58          | 0.0001     |
| Itgav       | integrin alpha V                                                          | 206456         | 1.61          | 0.0004     |
| Itgav5      | integrin alpha 5 (fibronectin receptor alpha)                            | 315346         | 3.47          | 0.00000001 |
| Itgb2       | integrin beta 2                                                           | 309664         | 3.44          | 0.0001     |
| Itgb7       | integrin, beta 7                                                          | 25713          | 3.86          | 0.002      |
| **C-type lectin receptors** |                                                                       |                |               |            |
| Clec4a1     | C-type lectin domain family 4, member a1, Dcir4                           | 362430         | 2.38          | 0.001      |
| Clec4a3     | C-type lectin domain family 4, member a3, Dcir3                           | 362431         | 3.00          | 0.0004     |
| Clec7a      | C-type lectin domain family 7, member a, Dectin 1                         | 502902         | 8.48          | 0.001      |
| Clec11a     | C-type lectin domain family 11, member a, Scgf                             | 29313          | 4.24          | 0.00000008 |
| **Syk and Syk-binding and intermediate partners** |                                                                       |                |               |            |
| Ezr**       | ezrin                                                                     | 54319          | 2.42          | 0.0001     |
| Lcp2        | lymphocyte cytosolic protein 2                                            | 155918         | 2.02          | 0.007      |
| Ptk2b       | PTK2B protein tyrosine kinase 2 beta                                      | 50646          | 2.54          | 0.0000002  |
| RhoG        | ras homolog gene family, member G (rho G)                                | 308875         | 1.54          | 0.0008     |
| RhoH        | ras homolog gene family, member H                                        | 305341         | 2.25          | 0.001      |
| Syk         | spleen tyrosine kinase                                                    | 25155          | 5.43          | 0.00004    |
| Vav1        | vav 1 guanine nucleotide exchange factor                                  | 25156          | 4.60          | 0.0001     |
| **NFκB genes and pathway** |                                                                       |                |               |            |
| Ikkb        | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase eta| 84351          | 1.60          | 0.0006     |
| Ikkbe       | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon | 363984     | 1.64          | 0.0001     |
| Nfkb1       | nuclear factor of kappa light polypeptide gene enhancer in B-cells 1      | 81736          | 1.88          | 0.002      |
| Nfkb2       | nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100 | 309452 | 1.98          | 0.00003    |
| **Cytokine and chemokine transcription** |                                                                       |                |               |            |
| Ccl2        | chemokine (C-C motif) ligand 2                                            | 24770          | 3.95          | 0.01       |
| Ccl7        | chemokine (C-C motif) ligand 7                                            | 287561         | 7.90          | 0.002      |
| Il1b        | interleukin 1 beta                                                        | 24494          | 5.17          | 0.002      |
| Ltb         | lymphotoxin beta (TNF superfamily, member 3)                              | 361795         | 3.77          | 0.0002     |
Table 4 Members of the Syk kinase pathway up-regulated in DA synovium compared with down-regulation in DA.F344(Cia5a) (Continued)

| Cell Proliferation |  |  |  |
|--------------------|----------------|----------------|----------------|
| Cdc2               | cell division cycle 2, G1 to S and G2 to M | 54237          | 24.16          | 0.000002 |
| Ccnb2              | cyclin B2      | 363088         | 22.95          | 0.000005 |
| Cks2               | CDC28 protein kinase regulatory subunit 2 | 498709         | 11.62          | 0.000006 |
| Prc1               | protein regulator of cytokinesis 1           | 308761         | 13.08          | 0.000002 |
| Spc24              | SPC24, NDC80 kinetochore complex component, homolog (S. cerevisiae) | 363028         | 14.72          | 0.000002 |

**Cytoskeletal changes**

| Actr3              | ARP3 actin-related protein 3 homolog (yeast) | 81732          | 1.88           | 0.0001  |
| Arpc4              | actin related protein 2/3 complex, subunit 4 | 297518         | 1.61           | 0.000005|
| Capzb              | capping protein (actin filament) muscle Z-line, beta | 298584         | 1.64           | 0.0003  |
| Coro1b             | coronin, actin-binding protein, 1B            | 29474          | 1.68           | 0.0001  |
| Myo9b              | myosin IX6                                    | 25486          | 1.93           | 0.000003|
| Tiam2              | T-cell lymphoma invasion and metastasis 2     | 308142         | 2.61           | 0.000003|
| Parva              | parvin, alpha                                 | 57341          | 2.19           | 0.000005|

**Reactive oxygen species production**

| Cyba                | cytochrome b-245, alpha polypeptide           | 79129          | 2.72           | 0.000001|
| Ncf1               | neutrophil cytosolic factor 1                | 114553         | 6.00           | 0.0001  |
| Ncf2               | neutrophil cytosolic factor 2                | 364018         | 4.75           | 0.0003  |
| Ncf4               | neutrophil cytosolic factor 4                | 500904         | 2.46           | 0.004   |
| Rac2¶              | ras-related C3 botulinum toxin substrate 2 (small GTP binding protein Rac2) | 366957         | 2.32           | 0.0007  |

Syk gene is underlined. * t-test; §=All four chemokine and cytokine listed genes are NFkB-inducible targets.
** Ezrin is also involved in cytoskeleton regulation.
¶ Rac also regulates NFkB activity, cytoskeleton and cell proliferation.

Figure 3 Syk pathway genes and Syk-regulated cellular processes. Genes, gene families and cellular processes with members expressed in increased levels in DA synovial tissues, and significantly reduced levels in DA.F344(Cia5a) congenics.
included the NRs Lxra, Pparg, Rev-erba, Rora, Thra, and Thrb (Table 5).

Scd1 was the gene with the most significantly increased expression in DA.F344(Cia5a) congenics with a 55-fold difference compared with DA (Tables 2 and 5). Scd1 has been shown to reduce cytokine levels and to have anti-inflammatory activity [19,20]. We have previously reported that Scd1 is expressed in significantly reduced levels in synovial tissues from rats with severe arthritis, and increased in the synovial tissues of yet another arthritis-protected congenic strain [14].

Adipoq and Timp3, which is an inhibitor of the TNFα converting enzyme (TACE), were two additional anti-inflammatory genes expressed in significantly increased levels (>10-fold) in DA.F344(Cia5a) (Table 5).

Scd1 and some of the other genes up-regulated in DA. F344(Cia5a) synovial tissues such as Adipoq, Cidea, Cd36, Fabp4, Gpd1, Lpl, Lpin1, Mgst1, Plin, Pck1, Slc2a4 and Srebf1, are known to be inducible by NRs (Table 2 and Table 5). These observations suggest that NRs were not only increased in expressed levels but also had increased activity in synovial tissues from DA.F344 (Cia5a) compared with DA.

Genes located within the Cia5a interval have significantly different expression levels

75 of the 7,925 genes expressed by all samples were located within the Cia5a interval. 21 of these 75 had increased expression in DA synovial tissues, and 11 were increased in the congenics. 14 of these 21 differentially expressed genes had ≥2-fold-difference. Sphk1 and Sectm1b were the genes contained within the Cia5a interval with the most significantly increased expression in DA (7.58 and 7.61-fold, respectively), while Itgb4 and Dlg1p1 and were those with the most significantly increased expression in DA.F344(Cia5a) congenics (2.77 and 2.85-fold, respectively) (Table 6). Additionally, four genes located within the Cia5a interval were expressed only or predominantly in DA synovium, while two other genes were expressed predominantly in DA.F344(Cia5a) (Table 6). It is conceivable that these differences in expression levels of genes located within the Cia5a interval

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**Table 5** Anti-inflammatory genes and nuclear receptors up-regulated in DA.F344(Cia5a) congenics

| Gene Symbol | Gene Name | Entrez Gene ID | Fold difference DA/Cia5a | p-value* |
|-------------|-----------|---------------|--------------------------|---------|
| **Anti-inflammatory and regulators of immune responses** |
| Scd1 | stearyl-Coenzyme A desaturase 1 | 246074 | 54.62 | 0.0001 |
| Timp3 | TIMP metallopeptidase inhibitor 3 | 25358 | 11.48 | 0.000000003 |
| Adipoq | adiponectin, C1Q and collagen domain containing | 246253 | 10.96 | 0.000003 |
| **Nuclear receptors and an interacting protein** |
| Rorc | retinoic X receptor gamma, Nr2b3 | 83574 | 5.39 | 0.0001 |
| Pparg | peroxisome proliferator-activated receptor gamma, Nr1c3 | 25664 | 5.32 | 0.000005 |
| **Re-erba** | Nrb1d1 | 252917 | 3.41 | 0.004 |
| **Arp1** | Nrb2f | 113984 | 2.45 | 0.0002 |
| **Nrip1** | nuclear receptor interacting protein 1 | 304157 | 2.35 | 0.00004 |
| **Thrb** | thyroid hormone receptor beta, Nr1a2 | 24831 | 2.07 | 0.002 |
| **Thra** | thyroid hormone receptor alpha, Nr1a1 | 81812 | 1.97 | 0.001 |
| **Ncor1** | nuclear receptor co-repressor 1 | 54299 | 1.89 | 0.003 |
| **Rora** | RAR-related orphan receptor alpha, Nr1f1 | 300807 | 1.88 | 0.0001 |
| **Lxra** | Liver X receptor alpha, Nr1h3 | 58852 | 1.52 | 0.0006 |

* t-test.
could be explained at least in part by a polymorphism in the 5′ untranslated region (UTR) that affects a transcription factor binding site in cis, thus affecting transcription efficiency, or a 3′ UTR polymorphism affecting mRNA stability.

Gene targets of microRNAs (miRNA) contained within the Cia5a interval were not differentially expressed

The Cia5a interval contains six predicted miRNAs. We considered the possibility that polymorphisms in one of those six miRNAs could account for the Cia5a effect on gene expression and arthritis severity. In that case, such polymorphism would affect the miRNA activity on the transcription of its target genes. Therefore, we look for the differential expression of targets of all six predicted miRNAs located within the Cia5a interval. A list of target and non-target genes was generated for each of the six miRNAs, but no significant over-representation of targets was detected (Figure 4), suggesting that polymorphisms affecting the expression or function of the miRNAs contained within the Cia5a interval are less likely to explain the differences in gene expression identified in this study.

Analyses of cell type specific genes suggests synovial tissue cellularity differences between DA and DA.F344(Cia5a) Congenics

13 genes known to be specifically expressed by the cell types of interest to this study were used to compare DA and DA.F344(Cia5a) synovial tissues (Additional file 3: Table S2). The expression levels of those genes suggested increased numbers of FLS (consistent with synovial hyperplasia), macrophages, dendritic cells (DC), neutrophils and T cells in the synovial tissues of DA, compared with congenics (Additional file 3: Table S2). No gene specific for B cells, NK cells or Tregs were among those differentially expressed between the two strains, suggesting that the number of these cells in the synovial tissues of these two strains was not significantly different.

Discussion

Disease severity and articular damage are associated with increased risk for disability, joint deformities and reduced life expectancy in patients with RA [21-23]. Yet, little is known about the genes implicated in the regulation of disease severity and articular damage genes in RA, and these genes could be the most relevant targets.

Table 6 Differentially expressed candidate genes located within the Cia5a interval on rat chromosome 10*

| Gene Symbol | Gene name | Entrez Gene ID | Difference | p-value $^\|^§ |
|-------------|-----------|----------------|------------|----------------|
| Increased in DA | | | | |
| Igsf7 | immunoglobulin superfamily, member 7 | 287813 | 4.42 | 0.00008 |
| Lgals3bp | lectin, galactoside-binding, soluble, 3 binding protein | 245955 | 3.85 | 0.000001 |
| RGD1309310 | similar to mKIAA0195 protein | 303677 | 3.89 | 0.000001 |
| Sectm1b | secreted and transmembrane 1B | 287884 | 7.61 | 0.000001 |
| Slec16a3 | solute carrier family 16, member 3 | 80878 | 5.36 | 0.0000005 |
| Sphk1 | sphingosine kinase 1 | 170897 | 7.58 | 0.0000003 |
| Increased in DA.F344(Cia5a) | | | | |
| Itgb4 | integrin beta 4 | 25724 | 2.85 | 0.0004 |
| RGD1311422 | similar to CG8841-PA | 287822 | 2.57 | 0.002 |
| RGD1561778 | similar to dendritic cell-derived immunoglobulin(Ig)-like receptor 1, DlgR1 | 303666 | 2.77 | 0.002 |
| Slec25a10 | solute carrier family 25, member 10 | 170943 | 2.15 | 0.004 |
| Expressed only or predominantly in DA | | | | |
| Cd300lf | CD300 molecule-like family, member f | 287818 | 6.0 | 0.0003 |
| Fdcr | ferredoxin reductase | 79122 | 6.1 | 0.0047 |
| Cd7 | CD7 molecule | 303747 | 6.0 | 0.0003 |
| Sectm1a | secreted and transmembrane 1A | 287885 | 6.1 | 0.0047 |
| Expressed only or predominantly in DA.F344(Cia5a) | | | | |
| Aanat | arylalkylamine N-acetyltransferase | 25120 | 0.7 | 0.0047 |
| Hnbp3 | hexaribonucleotide binding protein 3 | 287847 | 1.6 | 0.1 |

*List contains the most significantly differentially expressed genes; § t-test was used to compare means for fold-difference calculations and Fisher’s Exact test to compare frequencies. #not statistically significant.
for new therapies aimed at preserving the joint architecture and function.

We have previously identified Cia5a, a 20.6 Mb arthritis severity and joint damage regulatory locus, on rat chromosome 10 [10]. Cia5a co-localizes with other arthritis severity loci identified in other rodent models of arthritis such as oil-induced arthritis (Oia3) [24], and CIA in a DAXxACI intercross (Cia27) [25]. There have been no genome-wide association or linkage studies of disease severity and joint damage in RA, and therefore, it is unknown whether the Cia5a syntenic region on human chromosome 17q22-q25 harbors a severity or joint damage arthritis regulatory gene. However, the human 17q22-q25 region contains a locus previously linked with RA susceptibility [26]. In the present study we analyzed synovial tissues from DA rats, which develop severe arthritis (PLA) with pronounced synovial hyperplasia and cartilage and bone destruction, and synovial tissues from the DA.F344(Cia5a) congenics, which develop mild and non-erosive disease. These two strains share the same MHC and are genetically identical except for the presence of F344 alleles at the Cia5a interval, underscoring the magnitude of the effect of this single locus on clinical disease, on histologic joint damage [10] and on gene expression (present study). DA.F344(Cia5a) congenics had significantly reduced expression of genes previously implicated in RA pathogenesis, RA severity and articular damage, including Il1b, Il18, Mif, Mmp3 and Mmp14. These and other similarities between DA rats and RA synovial tissues’ gene expression, such as increased expression of chemokines, matrix proteins, adhesion molecules, mediators of innate immune responses, and others, underscore and further validate the potential clinical relevance of our model and discovery strategy.

We identified a new role for Cia5a on the regulation of the expression of members of the Syk pathway, where forty-seven genes directly or indirectly related to Syk activation were expressed in increased levels in DA, and significantly reduced levels in DA.F344(Cia5a) congenics. Syk is a tyrosine kinase that phosphorylates ITAM motifs in trans-membrane receptors or adaptors, and interacts with partners like Vav, PI3K and Slp76 [27]. Syk activation mediates signaling through several cell surface receptors, including those with significantly different levels in this study such C-lectin type receptors, Fcεr1g, Fcgr2a, Trem2, Tyrobp, integrins, and the T-cell receptor (TCR) (Figure 3). Resident and infiltrating inflammatory cells in the RA synovial pannus, such as mast cells, macrophages, B and T cells, express these Syk-activating receptors. These resident cells and infiltrating cells have been implicated in arthritis pathogenesis and joint damage, raising the possibility that part of their effect may be mediated by Syk-activating receptors.

Analyses of cell-specific genes suggested reduced numbers of macrophages, dendritic cells, neutrophils and T cells in the synovial tissues of congenics compared with DA, which is in agreement with our previous histologic analyses and might explain part, but not all of the differences in expression of Syk genes. Additionally, DA.F344 (Cia5a) congenics had significantly lower levels of the FLS-specific gene Cdh11, compatible with the reduced synovial hyperplasia that we have previously described.

Syk pathway members regulate several cellular processes implicated in arthritis pathogenesis and articular damage, ranging from the production of reactive oxygen species, NFκB activation and the transcription of pro-inflammatory mediators such as Il1b and Ccl2, to the cell proliferation required for the development of synovial hyperplasia, and actin cytoskeleton rearrangements [27]. NFκB activity is
regulated by Syk and by several other pathways including TLRs and cytokine receptors [28]. The NFκB pathway has a central role in the production of pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα, in the development of synovial hyperplasia and in disease severity [29-31]. Actin cytoskeleton rearrangements are also regulated by the Syk pathway [27], and are required for the migration of inflammatory cells into the synovial tissue, and for synovial cells and synovial tissue invasion and destruction of cartilage [13,32]. Therefore, our observations suggest that a gene located within the Cia5a interval is a new regulator of the expression of Syk pathway genes implicated in key processes in arthritis pathogenesis.

The precise mechanisms whereby Cia5a regulates the expression of Syk genes remain unclear, and might reflect differences in tissue cellularity, multiple cell-activating processes, or a polymorphism in transcription factor located within the Cia5a interval that affects transcription. Studies by our group of synovial tissues obtained from four different congenic strains yielded different results in gene expression [Brenner et al., manuscript in preparation] [12,14,33], suggesting that the Syk-regulatory effect of Cia5a is a specific observation, and not simply related cellularity differences or inflammation.

Syk has been recently implicated in arthritis pathogenesis and joint damage, and Syk-deficient mice are protected from autoantibody-induced erosive arthritis [34], and treatment with a SYK inhibitor significantly reduced disease severity and joint erosions and damage in collagen-induced arthritis [35]. Both the total and phosphorylated forms of SYK are expressed in increased levels in RA synovial tissues compared with osteoarthritis, and SYK inhibition reduced the expression of IL-6 and MMP-3 [17]. More importantly, the use of a SYK inhibitor significantly reduced disease activity in patients with RA [36], with 67%, 43% and 28% of patients achieving ACR20, ACR50 and ACR70, respectively, in a 3-month double-blind and placebo-controlled study [37]. Therefore, it is conceivable that the Syk pathway genes differentially expressed in this study could help identify patients more likely to benefit from therapy with SYK inhibitors. Additionally, Syk is critical to TNFα-induced responses [38], raising the possibility that the Syk pathway 47-gene signature could be used to predict increased TNFα activity prior to choosing a biologic therapeutic agent. Additionally, the increased expression of Syk pathway genes could identify patients at increased risk to develop erosive disease and could become a prognostic tool. Lastly, the Cia5a gene itself has the potential to become a new target for therapies aimed at reducing articular damage via inhibition of Syk pathway genes, including processes downstream from Syk such as NFκB.

While several genes with pro-inflammatory, proteolytic, innate immunity and inflamasome-related activity were expressed in reduced levels in DA.F344(Cia5a) congenics, groups of genes with known anti-inflammatory properties were expressed in increased levels in congenics. These genes included Timp3, Ptnm11, antagonists of reactive oxygen species (Cat, Gss, Sod1) and nuclear receptors. Nuclear receptors such as Lxra, Pparγ and Rora have been shown to interfere with NFκB and AP-1 activation [39-41], and to have anti-inflammatory and arthritis-suppressive properties [42-45]. Rxrg was another nuclear receptor expressed in significantly increased levels in DA.F344(Cia5a) congenics. While Rxrg itself has not been studied in the context of arthritis, it dimerizes with Lxra, Pparγ, and with Vdr, and is required for their anti-inflammatory activity. Additionally, several nuclear receptor-inducible genes, including the inflammation-suppressor Scd1 [20] were expressed in increased levels in the synovial tissues of the congenics. These observations suggest that not only nuclear receptor levels were increased, but also their activity. We have recently identified a similar nuclear receptor expression signature in another arthritis-protective congenic strain, DA.AC1(Cia25) [14], suggesting that this effect is not specific to the Cia5a locus, but more broadly correlates with preservation of both a normal synovial environment and articular architecture.

The gene with the most significantly increased expression in DA compared with congenics was Tnn (Tenascin N). While little is known about this secreted extracellular matrix glycoprotein, it has been implicated in cancer-associated angiogenesis [46], and in integrin-dependent cancer motility [47]. Another member of the tenascin family, Tenascin C (Tnc), was recently shown to be an endogenous activator of TLR4, an inducer of IL-6 and TNFα, and was required for joint damage in arthritic mice [48], suggesting that Tnn could have a function similar to Tnc in arthritis.

Lastly, we considered the possibility that a polymorphism in the 5’ UTR or 3’UTR region of the gene accounting for Cia5a could interfere with its transcription and/ or mRNA stability, respectively, leading to increased or reduced gene-specific mRNA levels. We looked for differentially expressed genes and genes preferentially expressed by only one of the strains and located within Cia5a as a clue to the above possibility. Thirty-eight genes met these criteria, and particularly the most significant sixteen genes are interesting candidates that will be studied in detail (Table 6).

**Conclusion**

In conclusion, in the present study we identified a pattern of gene expression regulated by Cia5a, which included several inflammatory mediators and 47 members of the Syk pathway. Levels of several mediators of arthritis
pathogenesis, synovial hyperplasia and articular damage were also reduced in DA.F344(Cia5a) congenics, underscoring the importance of the gene accounting for this locus. Increased expression of nuclear receptors correlated with joint preservation, and a new potential mediator of inflammation, Tmn, was identified for the first time in synovial tissues. Our observations suggest that the gene accounting for Cia5a has the potential to become an important new target for therapies aimed at preserving joint architecture free of damage, and reducing inflammation.

**Methods**

**Rats**

DA/BklArbNsi (DA) rats were originally purchased from Bantin & Kingman (Freemont, CA), maintained at the Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Disease, National Institutes of Health, and then transferred to the Feinstein Institute for Medical Research (FIMR; formerly North Shore-LIJ Research Institute, Nsi). DA.F344 (Cia5a) congenic rats were generated as previously described [9]. Briefly, a 20.6Mb interval from chromosome 10 from the arthritis-resistant F344 strain was introgressed into arthritis-susceptible DA rats through genotype-guided breeding (Figure 1A). This strategy selected for F344 alleles at the Cia5a interval while excluding donor genome contamination at other loci known to regulate arthritis [10,49]. Experiments were done with 8–12 week-old male rats homozygous at the congenic interval. All experiments were conducted under an Institutional Animal Care and Use Committee (IACUC)-approved protocol.

**PIA and tissue collection**

Male DA (n=6) and DA.F344(Cia5a) (n=8) congenic rats were anesthetized and injected intradermally with 150 μl of pristane (MP Bio, Solon, OH) divided into two injection sites at the base of the tail (day 0) [50]. Arthritis severity was assessed with a previously described 80-point scoring system [51]. Ankle synovial tissues were collected 21 days post-induction of arthritis.

**RNA extraction**

Total RNA was extracted from synovial tissues using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions and including a DNase treatment step. RNA was quantified and assessed for purity using the NanoDrop spectrophotometer (Rockland, DE). RNA integrity was verified with the BioAnalyzer 2100 (Agilent, Palo Alto, CA).

**Microarray**

All reagents and procedures were previously optimized for use with the Illumina Whole-Genome Expression platform [12]. Briefly, total RNA (200 ng) was amplified and biotinylated using the TotalPrep labeling kit (Ambion, Austin, TX). Each individual sample was hybridized to one individual array in the RatRef-12 Expression BeadChip (Illumina, San Diego, CA), which contains 22,522 probes covering 21,922 rat genes selected primarily from the NCBI RefSeq database (Release 16). Hybridization was done in Illumina IntelliHyb chambers, followed by washing and staining with Cy3-streptavidin. The BeadChip was scanned on a high-resolution Illumina BeadArray reader using a two-channel 0.8 μm resolution confocal laser scanner.

**cDNA synthesis and quantitative real-time PCR (qPCR) expression analysis**

Differences in the expression of selected genes from the microarray analyses were validated with qPCR. The qPCR conditions have been described elsewhere [12]. Briefly, total RNA (200 ng) from each sample was used for cDNA synthesis using Superscript III (Invitrogen). Primers and qPCR probes were designed to target the same exons as the corresponding Illumina RatRef-12 Expression BeadChip probes (Additional file 4: Table S1). We used Universal ProbeLibrary (Roche, Indianapolis, IN) and Taqman (ABI, Applied Biosystems, Foster City, CA) probes labeled with FAM at the 5’ end and TAMRA at 3’ end. Reactions were prepared in duplicates with Eurogentec qPCR MasterMix (San Diego, CA), and run on an ABI Prism 7700 thermocycler using SDS software version 1.9.1 (ABI). Ct (threshold cycle) values were adjusted for Gapdh in each sample (ΔCt). Expression levels (ΔΔCt) were compared using the t-test and a p-value ≤0.01 was considered significant. Fold-differences were calculated with the $2^{-ΔΔCt}$ method [52].

**MicroRNAs (miRNA)**

We considered the possibility that polymorphisms in a miRNA located within the Cia5a interval could account for the Cia5a effect on gene expression and arthritis severity. In that case, such polymorphism would affect the miRNA activity on the transcription of its target genes. Therefore, we looked for miRNAs mapping to the Cia5a interval using the miRBase [53]. Target genes for the miRNAs contained within the Cia5a interval were predicted with TargetScan [54]. Enrichment for differentially expressed predicted targets of miRNAs located within Cia5a was calculated using the Chi-square test with the Yates correction.

**Cellular subset gene expression signatures**

Differences in tissue resident and infiltrating cell populations can affect the interpretation of gene expression analyses. We looked for cell-specific genes using the
GNF Mouse GeneAtlas V3 (Affymetrix MOE430, GEO code GSE10246), a database containing gene expression information for 96 resting and stimulated mouse cell types and tissues [55,56], as well as the BioGPS website (www.biogps.org, Scripps Research Institute). The GNF Mouse GeneAtlas V3 did not include fibroblast-like synoviocytes (FLS) or regulatory T cells (Tregs). Therefore, additional non-redundant cell signature genes were obtained from the literature to represent FLS and Tregs [57-65]. We generated a list of genes specific for B cells, T cells, Treg cells, NK cells, FLS, dendritic cells, mast cells, macrophages and neutrophils. We next looked for those cell-specific genes within the list of genes differentially expressed between DA and DA.F344(Cia5a) congenics, as well as in the list of genes preferentially expressed, or only expressed in one strain and not in the other in order to gain insight into differences in cell populations.

**Microarray analysis and statistics**

Microarray fluorescence intensities were extracted using BeadStudio 2.0 (Illumina). Fluorescent intensities were background-subtracted and then normalized using the cubic spline algorithm. Normalized data were log2-transformed prior to all analyses. Probes consistently expressed in all arrays were included in the analyses. Genes with ≥1.5-fold difference in intensity between DA and DA.F344(Cia5a) and a t-test p-value ≤ 0.01 were considered differentially expressed and selected for pathway detection analyses using IPA 5.5.1 (Ingenuity Systems, Redwood City, CA), as well as public online databases (Ensembl, Genecards, Oncomine, BioGPS, Rat Genome Database) and literature search (Pubmed).

Strain-specific (genes only in one of the strains), or preferential strain (genes expressed in a higher percentage of rats of one strain, and in lower percentage of rats of the other strain) gene expression was determined with the Fisher’s exact test. Enrichment for biological functions and disease groups was determined with the IPA software and calculated using the Fisher’s exact test with the Benjamini-Hochberg correction and a cutoff p-value of ≤0.05. Enrichment for differentially expressed genes within specific cell subsets, or genes located within the Cia5a interval was calculated using the Fisher’s exact test. Non-normally distributed arthritis severity scores were compared using the Mann–Whitney test.

Funded by a Postdoctoral Fellowship Award from the New Jersey Chapter of the Arthritis Foundation to Dr. M. Brenner, and by the National Institutes of Health grants R01-AR46213, R01-AR052439 (NIAMS) and R01-AI54348 (NIAID) to Dr. P. Gulko.

**Additional files**

**Additional file 1**: Table S3. Functional categories related to angiogenesis and extra-cellular matrix turnover that were significantly down-regulated in DA.F344(Cia5a) synovium.

**Additional file 2**: Table S4. Functional categories related to pro-inflammatory signals, chemotaxis, and activation of myeloid cells that were significantly down-regulated in DA.F344(Cia5a) synovium.

**Additional file 3**: Table S2. Detection frequency and expression values of cell subset specific genes in DA and DA.F344(Cia5a) synovial tissues.

**Additional file 4**: Table S1. Primers and probes used for qPCR and the exons they targeted.

Competing interests

The authors have no competing financial interests to declare. The results presented in this manuscript are the basis for a recently submitted patent application.

**Authors’ contributions**

MB carried out the work with rats, including induction of arthritis, tissue dissection and all the steps in the microarray experiments, including a significant role in the analyses, interpretation of the results and manuscript writing. PSG conceived and designed the study and did the statistical and pathway analyses analysis, as well as the manuscript writing. Both authors read and approved the final manuscript.

Received: 20 August 2012 Accepted: 7 December 2012 Published: 19 December 2012

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Cite this article as: Brenner and Gulko: The arthritis severity locus Cia10 regulates the expression of inflammatory mediators including Syk pathway genes and proteases in pristane-induced arthritis. *BMC Genomics* 2012, 13:710.