EXTENDED REPORT

Rsk2 controls synovial fibroblast hyperplasia and the course of arthritis

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ABSTRACT

Objective Arthritis is a chronic inflammatory disease characterised by immune cell infiltration and mesenchymal cell expansion in the joints. Although the role of immune cells in arthritis is well characterised, the development of mesenchymal cell hyperplasia needs to be better defined. Here, we analysed the role of the ribosomal S6 kinase Rsk2, which we found to be highly activated in joints of patients with arthritis, in the development of mesenchymal cell hyperplasia.

Methods We genetically inactivated Rsk2 in the tumour necrosis factor (TNF)- α transgenic (TNFtg) mice, an animal model for human inflammatory arthritis. Clinical and histological signs of arthritis as well as molecular markers of inflammation and joint destruction were quantified. Fibroblast-like synoviocytes (FLS) were characterised in vitro and the effect of Rsk2 deletion on the pattern of gene expression was determined.

Results Rsk2 deficiency in TNFtg mice results in earlier and exacerbated inflammation as well as increased bone and cartilage destruction. The production of inflammatory cytokines, matrix metalloproteinases and osteoclastogenic molecules was significantly increased in vivo upon Rsk2 inactivation. Bone marrow deficient in Rsk2 could not transfer this phenotype, indicating that Rsk2 expression in mesenchymal cells controls the course of arthritis. Indeed, Rsk2 deficiency was associated with a more activated phenotype and higher proliferative capacity of FLS, thereby increasing cytokines and production of matrix proteinases.

Conclusions Rsk2 emerges as a key regulator of mesenchymal cell numbers in the joint and thereby could be targeted to control the inflammatory and tissue-destructive feature of joints in arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease leading to joint destruction.^{1 2} Although the initiating event is unknown, interaction between two cellular compartments, that is, haematopoietic cells and resident fibroblasts, is essential for disease development.^{3 4} RA is characterised by a massive influx of immune and inflammatory cells into joints.⁵ This process is governed by activation of resident fibroblasts secreting pro-inflammatory cytokines that sustain activation of inflammatory cells,⁶ matrix metalloproteinases (MMPs) that degrade the cartilaginous matrix and mediators of bone destruction, such as the receptor activator of NF- κ B ligand (RANKL), that locally support differentiation and activity of bone

resorbing osteoclasts.^{1 2 7–10} Importantly, fibroblast hyperplasia is a determinant factor contributing to severity and chronicity of RA.^{4 7 11} Indeed, in addition to its role in cartilage destruction, synovial hyperplasia is essential to maintain the local inflammation characterising the chronic course of RA. Mechanistically, tumour necrosis factor- α (TNF- α) stimulated RA fibroblasts would produce high amounts of pro-angiogenic factors and chemokines promoting neovascularisation, attracting and activating pro-inflammatory haematopoietic cells into the inflamed joint.^{4 7} Thus, understanding the control of synovial fibroblast numbers is essential for better defining arthritis and improving its treatment. However, factors regulating fibroblast hyperplasia in arthritic joints are poorly known.

The synovial hyperplasia in RA is likely resulting from increased resistance to apoptosis combined with enhanced proliferation of synovial fibroblasts. Indeed, one interesting but poorly understood aspect of synovial hyperplasia is that, although being activated by TNF- α and interleukin-1 (IL-1), fibroblasts are becoming independent of these cytokines as shown by their capacity to invade healthy joints when transferred to non-arthritic mice.¹² This suggests a key role of acquired epigenetic modifications in the persistence of the pathological behaviour of RA fibroblasts.^{7 13} Although not being oncogenic, resistance to apoptosis and increased proliferation clearly confer a transformed-like phenotype to RA fibroblasts.^{7 13} Importantly, inactivating mutations of the antiproliferative and pro-apoptotic oncogene p53 in the synovial fibroblasts of patients with RA have been consistently reported.^{14 15} Notably, inhibiting p53 activity is sufficient to transmit invasive properties to normal synovial fibroblasts.¹⁶ In agreement, while mice with genetic inactivation of the E3 ubiquitin ligase targeting p53 degradation synovioblasts are protected against collagen-induced arthritis, mice overexpressing synovioblasts develop spontaneous arthritis.^{17 18} As a consequence of p53 inactivation, RA fibroblasts become refractory to apoptosis due to a high level of Pi3 kinase activation. Interestingly, activation of Pi3K γ also governs synovial fibroblasts expansion.¹⁹

The 90 kDa ribosomal S6 kinase (Rsk2) is directly co-activated by extracellular signal-regulated kinase (ERK) and the Pi3 kinase-dependent kinase PDK1.^{20 21} Rsk2 is activated by TNF- α in various cells,²² including mesenchymal cells such as osteoblasts,²³ which suggests its implication in mediating

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TNF- α effects. Indeed, Rsk2 protects osteoblasts and osteocytes from TNF- α -induced apoptosis and thereby guards from TNF- α -induced systemic bone loss in arthritis.²³ Whether Rsk2 regulate the synovial fibroblast number in inflamed joints is unknown.

To test this hypothesis, we analysed the development of TNF-induced arthritis in Rsk2-deficient arthritic mice and characterised the phenotypical changes of arthritic synovial fibroblast lacking Rsk2.

MATERIALS AND METHODS

See online supplementary text.

RESULTS

Increased RSK activation in human inflamed arthritic synovium

We recently demonstrated the protective role of Rsk2 against TNF- α -induced systemic bone loss.²³ This observation made us comparing RSK activation between joints of patients with RA, inflamed (OAi) and non-inflamed osteoarthritis (OAni). An antibody characterising activated RSK1 and RSK2 revealed stronger reactivity in RA and OAi compared with OAni synovium (figure 1A) that was confirmed by double-blinded semiquantitative scoring (figure 1B). In fact, increased RSK activation occurred in lining and sublining layer of inflamed synovia (figure 1C, D). As this antibody cannot discriminate between RSK1 and RSK2, we stained metatarsal joints of transgenic mice overexpressing the human TNF- α (tumour necrosis factor transgenic (TNFtg) mice) and littermates lacking Rsk2 (TNFtg;Rsk2^{-/-}). The intense reactivity seen in inflamed joints of TNFtg mice was almost completely abrogated in the absence of Rsk2 (figure 1E). Thus, Rsk2 is highly activated in inflamed synovia of patients with RA and mice.

Early onset and increased severity of arthritis in Rsk2-deficient mice

Rsk2 being activated in inflamed joints of TNFtg mice, we compared clinical symptoms of arthritis between TNFtg mice and TNFtg;Rsk2^{-/-} littermates. Drastically increased paw swelling and decreased grip strength were observed in the absence of Rsk2. Remarkably, clinical symptoms already developed in 4-week-old TNFtg;Rsk2^{-/-} mice when no signs of arthritis were yet seen in TNFtg mice (figure 2A, B). Thus, an increased severity and earlier onset of TNF-mediated arthritis characterised Rsk2-deficient mice.

Histomorphometry of tarsal joints confirmed the clinical observations. Indeed, a drastically increased inflammation was seen on H&E-stained sections of 6-week-old TNFtg;Rsk2^{-/-} mice (figure 2C, D). Toluidine blue staining revealed an almost complete loss of proteoglycan in the articular cartilage of 6-week-old and 10-week-old TNFtg;Rsk2^{-/-} mice (figure 2E, F). Increased local bone destruction caused by increased numbers of bone resorbing osteoclasts was also measured in young and older TNFtg;Rsk2^{-/-} mice (figure 2G–I). Importantly, knees that are usually less affected in TNFtg mice were severely destroyed in 6-week-old TNFtg;Rsk2^{-/-} mice as indicated by an almost absent Safranin-O staining and of *Collagen-2 α* expression in areas where surface cartilage is normally present (figure 3A). Osteoclast-mediated bone destruction was also increased (figure 3A). Thus, inactivating Rsk2 resulted in severely accelerated, exacerbated and extended joint inflammation and destruction.

Increased cytokines and metalloproteinases production in TNFtg;Rsk2^{-/-} mice

To understand the molecular mechanism driving the exacerbation of arthritis, we analysed the expression of local mediators of joint destruction in the knees. Rsk2 deletion resulted in increased expression of mRNA encoding for the pro-inflammatory cytokines IL-1 β and IL-6 (figure 3B, C) and for the pro-osteoclastogenic factor RANKL while its decoy receptor osteoprotegerin (OPG) appeared unchanged compared with wild-type mice (figure 3D, E). Metalloproteinases *Mmp3* and *Mmp9* that mediate cartilage destruction were also drastically increased (figure 3F, G). *Mmps* being mainly produced by activated fibroblasts in inflamed joints of RA,³ we quantified the expression of *vimentin* and *integrin- α 1* (*Itga1*), two markers for fibroblastic mesenchymal cells. Both were upregulated, suggesting an expansion of the fibroblastic compartment in the inflamed joints lacking Rsk2 (figure 3H, I). Increased staining for cadherin-11, another specific marker of synovial fibroblasts²⁴ in the paws of TNFtg;Rsk2^{-/-} mice, supported this hypothesis (see online supplementary figure S1A). Anti-inflammatory mediators (TGF- β , IL-10 and IL-1-Ra) were also increased in the knees of TNFtg;Rsk2^{-/-} mice (figure 3J–L). Notably, TGF- β was the only cytokine already increased in joints of the Rsk2-deficient mice in the absence of TNF overexpression (figure 3J). At the cellular level, increased *CD11b* and a drastic *F4/80* augmentation indicated an increased macrophage maturation, and *CD11c* an increased dendritic cells accumulation (figure 4A–C). Increased *B220* and *CD3* or *CD4* suggested higher B-lymphocyte and T-lymphocyte recruitment (figure 4D–F). While no change in *RoryT* for Th17 cells was detected (data not shown), increased *FoxP3* suggested higher Tregs recruitment (figure 4G). Thus, increased synovial hyperplasia and recruitment of haematopoietic cells characterised inflamed joints of the TNFtg;Rsk2^{-/-} mice.

Measuring the levels of circulating IL-1 β , IL-6, hTNF- α and *Mmp3* confirmed their increase in TNFtg;Rsk2^{-/-} mice (figure 4H–K). These changes were not seen in Rsk2-deficient mice without TNF overexpression, indicating that Rsk2 could act downstream of TNF. Interestingly, no increased circulation of anti-inflammatory cytokines including IL-10, IL-13 and TGF- β was detected (data not shown and figure 4L). In addition, while no changes in the splenic T cell and B cell populations was observed by flow cytometry, all myeloid populations, namely *CD11b*⁺ monocytes, *F4/80*⁺ macrophages, *Ly-6G*⁺ neutrophils and *CD11c*⁺ dendritic cells, were increased in TNFtg;Rsk2^{-/-} mice (see online supplementary figure S2). Thus, higher local levels of pro-inflammatory cytokines resulted in an increased systemic inflammation in TNFtg;Rsk2^{-/-} mice.

Rsk2-deficient haematopoietic cells do not sensitise TNFtg mice to arthritis

The TNF transgene is not expressed in haematopoietic but rather in the mesenchymal compartment.²⁵ Nevertheless, exacerbated arthritis could result from an increased reactivity of Rsk2-deficient haematopoietic cells to TNF. Therefore, we performed adoptive transfer of Rsk2-deficient or wild-type bone marrow cells into irradiated TNFtg mice. Clinical observations and histomorphometry revealed no differences between both groups (see online supplementary figure S3). Thus, the arthritis severity is independent of Rsk2 expression in haematopoietic cells.

Unchanged cytokine and metalloproteinase expression in Rsk2-deficient synovial fibroblasts

We next isolated synovial-like fibroblasts (FLS) from the paws of TNFtg and TNFtg;Rsk2^{-/-} mice (see online supplementary

Figure 1 RSK is activated in human and murine inflamed arthritic synovium. (A) Immunostaining for phospho-RSK1/2 of human synovium from patients with rheumatoid arthritis (RA), inflamed osteoarthritis (OAi) and non-inflamed osteoarthritis (OAni), two magnifications of representative staining (20× and 40×) are shown as well as a staining with the isotype control (isotype). All sections were counterstained with haematoxylin. (B) Expression score of the immunostaining performed by two blinded investigators on the full section, (C) in the lining and (D) in the sublining layer of the synovium. (E) Immunostaining of phospho-Rsk1/2 of the tarsal joint of 6-week-old tumour necrosis factor transgenic (*TNFtg*) and *TNFtg;Rsk2*^{-/-} mice.

figures S1) to determine whether expression of pro-inflammatory cytokines and Mmps were increased in FLS lacking *Rsk2*. The relative expression of the osteoclastogenic cytokines *Rankl* and *OPG*, of the pro-inflammatory cytokines *IL-6*, *TNF* and chemokine *KC*, of *Mmp3*, *Mmp9*, *Mmp13* and of *Vimentin*, were unaffected as were the secretion of *IL-6*, *KC*, *OPG* and *TNF* after 24 h of culture (see online supplementary figure S4). Thus, the in vivo increase of pro-inflammatory cytokines and metalloproteinases would rather be a consequence of fibroblast hyperplasia.

Increased proliferation of *Rsk2*-deficient synovial fibroblasts

We next compared the growth of FLS isolated from *TNFtg;Rsk2*^{-/-} and *TNFtg* mice. The cumulative cell number and cell growth at early passage (passage 4) indicated a significantly increased cell growth following *Rsk2* deletion (figure 5A, B). Apoptosis as shown by TUNEL staining and cell viability tested by MTT assay (see online supplementary figure S5) were unaffected. Fluorescence-activated cell sorting (FACS) analysis of eFluor marked fibroblasts confirmed the increased proliferation of the *TNFtg;Rsk2*^{-/-} FLS as demonstrated by the significant decreased

Figure 2 Early onset and increased severity of arthritis in tumour necrosis factor transgenic (*TNF*tg);*Rsk2*^{-/-} mice. Clinical assessment of paw swelling (A) and grip strength (B) in 4-week-old 11-week-old wild type (wt), *Rsk2*-deficient (*Rsk2*^{-/-}), human *TNF*- α transgenic (*TNF*tg) and *Rsk2*-deficient human *TNF*- α transgenic (*TNF*tg;*Rsk2*^{-/-}) mice. Representative histology of the tarsal joints of 6-week-old *TNF*tg and *TNF*tg;*Rsk2*^{-/-} mice: (C) H&E staining (the arrows point to inflamed tissue) and (D) histomorphometric analysis of the inflammation area at 6 and 10 weeks of age; (E) toluidine blue staining for proteoglycan (the arrows point to unstained cartilage) and (F) histomorphometric analysis of the proportion of cartilage loss; (G) tartrate-resistant acid phosphatase (TRAP) staining for osteoclasts, histomorphometric analysis of (H) the erosion area and (I) of the numbers of TRAP-positive cells (osteoclasts).

mean fluorescence intensity at all time of the culture (figure 5C, D). The neutralising anti-TNF antibody infliximab did not affect cell growth, demonstrating that this increased proliferation was TNF- α independent (figure 5E, F). To confirm this hypothesis, we isolated FLS from *Rsk2*-deficient mice and wild-type counterparts. Again, *Rsk2* inactivation resulted in increased proliferation of the cells lacking *Rsk2* (figure 5G, H and online supplementary figure S5D,E). Thus, *Rsk2* acts as a TNF-independent negative regulator of synovial fibroblast proliferation.

Gene expression profiling reveals a cell autonomous increased activation of *Rsk2*-deficient synovial fibroblasts.

We then performed Affymetrix-based gene expression analyses to better compare the *TNF*tg;*Rsk2*^{-/-} to *TNF*tg cells. We identified 379 downregulated and 144 upregulated genes with a fold change (FC) higher than 2. With high-performance Chip data analysis (HPCDA), we reduced the list to genes with a HPCDA score superior to 1000 and an absolute signal-log-ratio superior to 2 (ie, FC>4). This identified 21 downregulated and 7

Figure 3 Increased inflammation, cartilage and bone destruction in the knee of tumour necrosis factor transgenic (TNFtg);*Rsk2*^{-/-} mice. (A) Safranin-O staining, in situ hybridisation for *Col2a* (collagen2a) and tartrate-resistant acid phosphatase (TRAP) staining of sections from knees of 6-week-old mice of the indicated genotypes. Quantitative PCR analysis of (B) interleukin-1β (*IL-1β*) and (C) *IL-6* as markers of inflammation; (D) *RANKL* and (E) osteoprotegerin (*OPG*) as markers for osteoclastogenesis, (F) matrix metalloproteinases *Mmp-3* and (G) *Mmp-9* as regulators of cartilage destruction, (H) *Vimentin* and (I) *Integrin-α1* (*Itga1*) as markers for synovial fibroblasts, (J) *TGF-β*, (K) *IL-10* and (L) *IL-1Ra* as anti-inflammatory markers in the knees of 6-week-old mice of the indicated genotypes (n≥5). Values represent means±SEM. All statistical analysis were compared with the TNFtg;*Rsk2*^{-/-} mice (*) excepted (#) compared with *Rsk2*^{-/-} mice.

Figure 4 Increased systemic and local inflammation in tumour necrosis factor transgenic (*TNFtg*);*Rsk2*^{-/-} mice. Q-PCR analysis of the expression of markers for (A) monocytes: *CD11b*, (B) macrophages: *F4/80*, (C) dendritic cells: *CD11c*, (D) B cells: *B220*, (E) T cells: *CD3*, (F) T helper cells: *CD4* and (H) Tregs: *FoxP3* in the knee of 6-week-old mice of the indicated genotype. ELISAs quantification of the levels of circulating (H) interleukin-1β (IL-1β), (I) IL-6, (J) hTNFα, (K) MMP3 and (L) TGF-β in 6-week-old mice of the indicated genotypes (n=7–11). All statistic analysis were compared with *TNFtg;Rsk2*^{-/-} mice.

upregulated hits with high probability to be differentially regulated (figure 6A and online supplementary table S2).

Quantitative PCR confirmed the increased expression of *Ptgs2*, and the decrease of *Sfrp2*, *Prep*, *Nov*, *Tlr2* and *Adam8* but not *Ccl11* in *TNFtg;Rsk2*^{-/-} FLS (figure 6B). Thus, upregulation of *Ptgs2* encoding *Cox2* and downregulation of genes potentially limiting joint destruction and inflammation such as *Prep*, *Adam8*, *Nov* and of *Prp4*, a known positive regulator of synovial fibroblast hyperplasia, confirmed an increased activation of *Rsk2*-deficient FLS.

DISCUSSION

Here, we identify *Rsk2* as a kinase preventing TNF-induced synovial hyperplasia, thereby protecting against inflammation-induced joint destruction.

Synovial hyperplasia is a central but poorly understood feature of chronic inflammatory arthritis. It provides continuous supply of inflammatory cytokines and chemokines that creates a local inflammatory milieu attracting immune cells to the

diseased joint.^{3 4 6 7} It is also a source of matrix proteinases and osteoclastogenic molecules, thereby linking arthritis and tissue destruction.²⁶ *Rsk2* activation that limits synovial hyperplasia appeared surprisingly increased in inflamed arthritic synovia compared with non-inflamed. This suggests that *Rsk2* is part of a negative regulatory loop that control overshooting of pro-inflammatory responses as it also did against TNF-induced apoptosis in osteoblasts.

Synovial hyperplasia is an accumulation of resident fibroblastic mesenchymal cells and macrophages. Although, the increased maturation of macrophages as a known source of pro-inflammatory cytokines most likely participates in the increased joint inflammation, our bone marrow transfer experiment excluded the macrophages as a cell autonomous cause for arthritis exacerbation. This conclusion also stands for B and T cells. Moreover, no signs of increased cytokines production by inflammatory Th1 cells (IL-2 and IFN-γ), nor increase of Th17 cells, were found. We rather found increased *FoxP3* expressing regulatory T cells most likely as a consequence of the local increased

Figure 5 Increased proliferation of tumour necrosis factor transgenic (*TNFtg*);*Rsk2^{-/-}* derived fibroblast-like synoviocytes. (A) Cumulative cell numbers and (B) cell count at passage 4 of *TNFtg* and *TNFtg;Rsk2^{-/-}* synoviocytes in culture. (C) Flow cytometric analysis of eFluor staining at day 2 of the culture. (D) Quantification of the kinetic of eFluor decay in the culture. Effect of infliximab on the growth of *TNFtg* cells (E) and *TNFtg;Rsk2^{-/-}* cells (F). (G) Cumulative cell numbers and (H) cell count at passage 4 of wild type (wt) and *Rsk2^{-/-}* synoviocytes in culture.

TGF- β expression. These cells could protect against RA;²⁷ however, this protection is usually not observed in patients due to impaired functionality of the cells²⁸ that may also occur in our mice.

Several observations rather point to the dominant role of the fibroblasts in the exacerbation of arthritis: first, histology revealed an increased number of cells expressing the marker of fibroblast-like synoviocytes cadherin-11²⁹ in the joints of *TNFtg;Rsk2^{-/-}* mice; second, the expression of the human TNF, known to be produced by transgenic mesenchymal cells,²⁵ was also increased in the knee in the absence of *Rsk2*. Finally, quantitative PCRs demonstrated increased expression in the inflamed knees lacking *Rsk2* of genes such as MMPs, IL-6, integrin- α 1 and vimentin that are mainly expressed by activated fibroblasts.⁷

The mechanism underlying the aggressive behaviour of fibroblasts during arthritis has clearly been linked to their enhanced invasive capacity.^{12 16} In contrast, the mechanism governing mesenchymal cells hyperplasia in synovium is still hypothetical but is believed to result from an imbalance between proliferation and apoptosis.³ Indeed, enhanced resistance to apoptosis due to epigenetic changes, inactivating mutations of tumour-suppressor

genes such as p53,^{14 15} increased expression of heat shock protein 70³⁰ and downregulation of the E3 ubiquitin ligase synoviolin¹⁷ have been proposed as mechanisms involved in fibroblast expansion in arthritis.^{26 31} However, our data excluded that an increased resistance to apoptosis could explain the more severe synovial hyperplasia in *Rsk2*-deficient arthritic mice. In addition, in agreement with others,^{22 32} who suggested an antiapoptotic function for *Rsk2* in mesenchymal cells, we previously reported increased apoptosis of c-Fos-transformed osteoblasts lacking *Rsk2*³³ and even the increased sensitivity of *Rsk2*-deficient osteoblasts to TNF-induced apoptosis in vitro.²³

The uncontrolled proliferation of synovial fibroblasts during inflammatory arthritis is still poorly understood. The kinase *Rsk2*, which acts downstream of the PI3K and Erk signalling pathways, seems to represent a key checkpoint for synovial fibroblasts proliferation. This conclusion is based upon three independent assays that all showed a cell autonomous increased growth capacity of *Rsk2*-deficient fibroblasts in vitro. Surprisingly, *Rsk2* blockade of osteoblast differentiation is independent of proliferation, while *Rsk2*-deficient c-Fos-transformed osteoblasts display decreased proliferation.^{23 33} Although we do not know the molecular basis of these differences, *Rsk2* function in cells of the same phylogenetic origin clearly depends on their lineage commitment and on the pathology affecting them.

Although TNF activates *Rsk2* in FLS (see online supplementary figure S6F), the increased proliferation of fibroblasts lacking *Rsk2* is independent of TNF as shown by the addition of neutralising antibodies against TNF. These data corroborate the reported properties of fibroblasts isolated from inflamed joints in RA that acquire a transformed-like phenotype in response to chronic inflammation and become independent of further inflammatory stimulation.^{3 4 12 16 34} Importantly, we observed increased proliferation in *Rsk2*-deficient FLS that did not over-express TNF. Notably, high level of TGF- β expression was found in the knees of *Rsk2^{-/-}* mice. Although the role of TGF- β in RA is controversial, it can act in combination with TNF- α , as a pro-inflammatory cytokine in FLS, support osteoclastogenesis and promote synovium hyperplasia.³⁵ Whether the increased TGF- β is driving the increased proliferative capacity of FLS lacking *Rsk2* remains to be tested.

Synovial hyperplasia is an important factor in mobilising inflammatory cells from the bone marrow and fostering their migration to peripheral sites. Indeed, we found increased numbers of splenic myeloid cells in *TNFtg;Rsk2^{-/-}* mice compared with *TNFtg* littermates, but no significant differences in the lymphoid cells. These increased frequencies of myeloid cells seem based on the enhanced pool of TNF-producing fibroblasts as suggested by the TNF-dependent increased frequency of CD11b^{high} cells in the circulation and secondary lymphatic organs.^{36 37}

Aside from attracting inflammatory cells, synovial hyperplasia also negatively affects bone and cartilage homeostasis in arthritis due to increased production of Mmps and of osteoclastogenic cytokines like RANKL and TNF. Although our in vitro phenotyping of isolated fibroblasts demonstrated similar expression of cytokines and Mmps when equal numbers of cells were plated, the comparison between the gene expression profiles clearly confirmed our hypothesis of a cell autonomous increased activation of the fibroblasts lacking *Rsk2*. We clearly identified misregulation of genes that would increase the aggressive phenotype of inflammatory synovial fibroblasts, for instance, decreased expression of secreted frizzled-related protein (*Sfrp*)-2, a potential Wnt antagonist. In agreement with the transformed-like phenotype of arthritic synovial fibroblasts, *Sfrp2* represses

Basic and translational research

Figure 6 Gene expression profiling of isolated fibroblast-like synoviocytes isolated from the paws of tumour necrosis factor transgenic (*TNFTg*); *Rsk2*^{-/-} mice compared with *TNFTg*. (A) Heat map of the differentially regulated genes with a high-performance Chip data analysis (HPCDA) score >1000 and an absolute signal log ratio (SRL)>2. (B) Q-PCR validation of differentially regulated genes (n=6 independent isolations for each genotype).

invasiveness and proliferation of tumoral cells.^{38 39} *Nov*, a positive regulator of chondrogenesis,⁴⁰ was also clearly downregulated. Interestingly, among the most differentially regulated targets were genes encoding for known regulators of inflammation, synovial hyperplasia or joint destruction such as downregulation of *Adam8*, *Prelp* and *Prg4* and upregulation of *Ptgs2* and *Cadherin11*. *Adam8*, which is a metalloproteinase induced by TNF acting as a sheddase for the TNF receptor I thereby limiting TNF activity,⁴¹ has the ability to limit leucocyte recruitment and inflammation.⁴² *Prelp*, an extracellular matrix protein found in the synovial fluid of patients with RA, limits inflammation and complement activation-mediated cartilage attack and represses osteoclast differentiation.^{43 44} *Prg4* encodes for Lubricin, a protein essential for the maintenance of articular cartilage and a negative regulator of synovial hyperplasia, thereby protecting against arthritis-induced joint destruction.^{45–47} *Ptgs2* is encoding for the central mediator of inflammation in arthritis, Cox2.⁴⁸ Finally, *Cadherin11* synergizes with TNF and IL1- β to positively regulate synovial hyperplasia, inflammation and joint destruction in arthritis.^{29 49 50} Thus, *Rsk2* regulates a number of pivotal genes in the pathogenesis of arthritis linked to the activated status of fibroblasts in the joint.

In summary, we identified *Rsk2* as a new key regulator of inflammation-induced synovial fibroblast hyperplasia that blunts their proliferation and activation, thereby downregulating in situ cytokine production and the resulting influx of haematopoietic cells into the joints. Hence, activating ERK/*Rsk2* could be a therapeutic option to interfere with synovial fibroblast aggressiveness in arthritis, a central pathogenic feature of chronic inflammatory joint disease in human.

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Contributors J-PD, AJH, GS and UG designed the experiments, analysed the data and wrote the article. AD and CB designed, performed and analysed the experiments. BG, SB and BS performed FACS analysis. JRG and AG generated and analysed the Affymetrix data. MS performed the in situ hybridisation.

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REFERENCES

- McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011;365:2205–19.
- Walsh NC, Gravalles EM. Bone remodeling in rheumatic disease: a question of balance. *Immunol Rev* 2010;233:301–2.
- Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev* 2010;233:233–55.

- 4 Neumann E, Lefevre S, Zimmermann B, *et al.* Rheumatoid arthritis progression mediated by activated synovial fibroblasts. *Trends Mol Med* 2010;16:458–68.
- 5 McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 2007;7:429–42.
- 6 Barone F, Nayar S, Buckley CD. The role of non-hematopoietic stromal cells in the persistence of inflammation. *Front Immunol* 2012;3:416.
- 7 Bottini N, Firestein GS. Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. *Nat Rev Rheumatol* 2013;9:24–33.
- 8 Goldring SR, Purdue PE, Crotti TN, *et al.* Bone remodelling in inflammatory arthritis. *Ann Rheum Dis* 2013;72:ii52–5.
- 9 Gravalles EM, Manning C, Tsay A, *et al.* Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. *Arthritis Rheum* 2000;43:250–8.
- 10 Shigeyama Y, Pap T, Kunzler P, *et al.* Expression of osteoclast differentiation factor in rheumatoid arthritis. *Arthritis Rheum* 2000;43:2523–30.
- 11 Muller-Ladner U, Pap T, Gay RE, *et al.* Mechanisms of disease: the molecular and cellular basis of joint destruction in rheumatoid arthritis. *Nat Clin Pract Rheumatol* 2005;1:102–10.
- 12 Lefevre S, Knedla A, Tennie C, *et al.* Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat Med* 2009;15:1414–20.
- 13 Karouzakis E, Gay RE, Gay S, *et al.* Epigenetic control in rheumatoid arthritis synovial fibroblasts. *Nat Rev Rheumatol* 2009;5:266–72.
- 14 Firestein GS, Echeverri F, Yeo M, *et al.* Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. *Proc Natl Acad Sci USA* 1997;94:10895–900.
- 15 Reme T, Travaglio A, Gueydon E, *et al.* Mutations of the p53 tumour suppressor gene in erosive rheumatoid synovial tissue. *Clin Exp Immunol* 1998;111:353–8.
- 16 Pap T, Aupperle KR, Gay S, *et al.* Invasiveness of synovial fibroblasts is regulated by p53 in the SCID mouse in vivo model of cartilage invasion. *Arthritis Rheum* 2001;44:676–81.
- 17 Amano T, Yamasaki S, Yagishita N, *et al.* Synoviolin/Hrd1, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy. *Genes Dev* 2003;17:2436–49.
- 18 Yamasaki S, Yagishita N, Sasaki T, *et al.* Cytoplasmic destruction of p53 by the endoplasmic reticulum-resident ubiquitin ligase 'Synoviolin'. *EMBO J* 2007;26:113–22.
- 19 Hayer S, Pundt N, Peters MA, *et al.* PI3Kgamma regulates cartilage damage in chronic inflammatory arthritis. *FASEB J* 2009;23:4288–98.
- 20 Pearce LR, Komander D, Alessi DR. The nuts and bolts of AGC protein kinases. *Nat Rev Mol Cell Biol* 2010;11:9–22.
- 21 Romeo Y, Zhang X, Roux PP. Regulation and function of the RSK family of protein kinases. *Biochem J* 2012;441:553–69.
- 22 Peng C, Cho YY, Zhu F, *et al.* RSK2 mediates NF- κ B activity through the phosphorylation of I κ B α in the TNF-R1 pathway. *FASEB J* 2010;24:3490–9.
- 23 Bohm C, Derer A, Axmann R, *et al.* RSK2 protects mice against TNF-induced bone loss. *J Cell Sci* 2012;125:60–71.
- 24 Valencia X, Higgins JM, Kiener HP, *et al.* Cadherin-11 provides specific cellular adhesion between fibroblast-like synoviocytes. *J Exp Med* 2004;200:1673–9.
- 25 Keffer J, Probert L, Cazlaris H, *et al.* Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 1991;0:4025–31.
- 26 Meinecke I, Rutkauskaitė E, Gay S, *et al.* The role of synovial fibroblasts in mediating joint destruction in rheumatoid arthritis. *Curr Pharm Des* 2005;11:563–8.
- 27 Zaiss MM, Frey B, Hess A, *et al.* Regulatory T cells protect from local and systemic bone destruction in arthritis. *J Immunol* 2010;184:7238–46.
- 28 Gol-Ara M, Jadidi-Niaragh F, Sadria R, *et al.* The role of different subsets of regulatory T cells in immunopathogenesis of rheumatoid arthritis. *Arthritis* 2012;2012:805875.
- 29 Lee DM, Kiener HP, Agarwal SK, *et al.* Cadherin-11 in synovial lining formation and pathology in arthritis. *Science* 2007;315:1006–10.
- 30 Schett G, Redlich K, Xu Q, *et al.* Enhanced expression of heat shock protein 70 (hsp70) and heat shock factor 1 (HSF1) activation in rheumatoid arthritis synovial tissue. Differential regulation of hsp70 expression and hsf1 activation in synovial fibroblasts by proinflammatory cytokines, shear stress, and antiinflammatory drugs. *J Clin Invest* 1998;102:302–11.
- 31 Maciejewska-Rodrigues H, Karouzakis E, Strietholt S, *et al.* Epigenetics and rheumatoid arthritis: the role of SENP1 in the regulation of MMP-1 expression. *J Autoimmun* 2010;35:15–22.
- 32 She QB, Ma WY, Zhong S, *et al.* Activation of JNK1, RSK2, and MSK1 is involved in serine 112 phosphorylation of Bad by ultraviolet B radiation. *J Biol Chem* 2002;277:24039–48.
- 33 David JP, Mehic D, Bakiri L, *et al.* Essential role of RSK2 in c-Fos-dependent osteosarcoma development. *J Clin Invest* 2005;115:664–72.
- 34 Han Z, Boyle DL, Shi Y, *et al.* Dominant-negative p53 mutations in rheumatoid arthritis. *Arthritis Rheum* 1999;42:1088–92.
- 35 Gonzalo-Gil E, Galindo-Izquierdo M. Role of transforming growth factor-beta (TGF) beta in the physiopathology of rheumatoid arthritis. *Rheumatol Clin* 2014;10:174–79.
- 36 Zsembery A, Fortenberry JA, Liang L, *et al.* Extracellular zinc and ATP restore chloride secretion across cystic fibrosis airway epithelia by triggering calcium entry. *J Biol Chem* 2004;279:10720–9.
- 37 Yao Z, Li P, Zhang Q, *et al.* Tumor necrosis factor-alpha increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. *J Biol Chem* 2006;281:11846–55.
- 38 Esteve P, Bovolenta P. The advantages and disadvantages of sfrp1 and sfrp2 expression in pathological events. *Tohoku J Exp Med* 2010;221:11–17.
- 39 Chung MT, Lai HC, Sytwu HK, *et al.* SFRP1 and SFRP2 suppress the transformation and invasion abilities of cervical cancer cells through Wnt signal pathway. *Gynecol Oncol* 2009;112:646–53.
- 40 Ouellet V, Siegel PM. CCN3 modulates bone turnover and is a novel regulator of skeletal metastasis. *J Cell Commun Signal* 2012;6:73–85.
- 41 Bartsch JW, Wildeboer D, Koller G, *et al.* Tumor necrosis factor-alpha (TNF-alpha) regulates shedding of TNF-alpha receptor 1 by the metalloprotease-disintegrin ADAM8: evidence for a protease-regulated feedback loop in neuroprotection. *J Neurosci* 2010;30:12210–18.
- 42 Dominguez-Luis M, Lamana A, Vazquez J, *et al.* The metalloprotease ADAM8 is associated with and regulates the function of the adhesion receptor PSGL-1 through ERM proteins. *Eur J Immunol* 2011;41:3436–42.
- 43 Happonen KE, Furst CM, Saxne T, *et al.* PRELP protein inhibits the formation of the complement membrane attack complex. *J Biol Chem* 2012;287:8092–100.
- 44 Rucci N, Rufo A, Alamanou M, *et al.* The glycosaminoglycan-binding domain of PRELP acts as a cell type-specific NF- κ B inhibitor that impairs osteoclastogenesis. *J Cell Biol* 2009;187:669–83.
- 45 Waller KA, Zhang LX, Elsaid KA, *et al.* Role of lubricin and boundary lubrication in the prevention of chondrocyte apoptosis. *Proc Natl Acad Sci USA* 2013;110:5852–7.
- 46 Coles JM, Zhang L, Blum JJ, *et al.* Loss of cartilage structure, stiffness, and frictional properties in mice lacking PRG4. *Arthritis Rheum* 2010;62:1666–74.
- 47 Rhee DK, Marcelino J, Baker M, *et al.* The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. *J Clin Invest* 2005;115:622–31.
- 48 Hochberg MC. New directions in symptomatic therapy for patients with osteoarthritis and rheumatoid arthritis. *Semin Arthritis Rheum* 2002;32:4–14.
- 49 Chang SK, Noss EH, Chen M, *et al.* Cadherin-11 regulates fibroblast inflammation. *Proc Natl Acad Sci USA* 2011;108:8402–7.
- 50 Kiener HP, Niederreiter B, Lee DM, *et al.* Cadherin 11 promotes invasive behavior of fibroblast-like synoviocytes. *Arthritis Rheum* 2009;60:1305–10.

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