Mod Rheumatol (2005) 15:19–27
DOI 10.1007/s10165-004-0358-6

REVIEW ARTICLE

Sakae Tanaka

Intracellular signal transduction pathways: good therapeutic targets for joint destruction in rheumatoid arthritis

Abstract Preventing joint destruction is one of the most challenging issues in treating patients with rheumatoid arthritis (RA), and I propose that intracellular signaling pathways in osteoclasts and synovial fibroblastic cells (SFCs) can be good therapeutic targets. Osteoclasts are primarily involved in the bone destruction in RA joints, and SFCs support osteoclast differentiation and activation by producing various proinflammatory cytokines including receptor activator of NF-κB ligand (RANKL), the osteoclast differentiation factor belonging to the tumor necrosis factor-α superfamily. Suppressing c-Src pathways by adenovirus vector-mediated C-terminal Src family kinase (Csk) gene or Ras/extracellular-regulating kinase (ERK) pathways by introducing dominant negative Ras (Ras^{DN}) adenovirus reduced osteoclastic bone resorption as well as the abnormal proliferation and interleukin-6 production of SFCs, and the local injection of these viruses ameliorated the joint destruction in adjuvant arthritis rats. Moreover, chondrogenic differentiation of SFCs could be induced by stimulating activin receptor-like kinase 3 pathways.

Key words Adenovirus · Osteoclast · Rheumatoid arthritis (RA) · Synovial fibroblast cells (SFCs)

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder with an unknown etiology characterized by the invasive synovial hyperplasia leading to the progressive joint destruction.[1] Radiographic studies have shown that the bone erosion in RA begins at the early stage of the disease, and gradually exacerbates. Bone erosion results in the severe deformity of the affected joints and impairs the normal activity and the quality of life of the RA patients, and hereby, preventing such devastating states is one of the most challenging issues in treating them. Because the exact etiology and pathology of RA remains unknown, most treatments of RA just treat symptoms of the disease. Non-steroidal anti-inflammatory drugs, including cyclooxygenase 2 inhibitors, have been prescribed to reduce the painful symptoms of the disease, but they have little effect on stopping the progression of the joint destruction. Recent studies have revealed that some disease-modifying antirheumatic drugs and biological agents such as anti-tumor necrosis factor (TNF)-α antibody ameliorate the progression of the joint destruction in RA.[2] However, the bone-protective effect of these reagents is limited in most cases, and their long-term effects have not been established yet. Moreover, the prolonged usage of these medicines sometimes causes severe side effects. Therefore, novel therapeutic interventions specifically targeting the joint destruction in RA are greatly expected.

Proliferating synovium produces an elevated amount of proinflammatory cytokines interleukin (IL)-1, IL-6, IL-17, and TNF-α, and matrix-degenerating enzymes matrix metalloproteinases and cathepsins, which are involved in the bone and cartilage destruction.[1,3] Considerable data have demonstrated that synovial fibroblastic cells (SFCs), type B synovial cells with fibroblastic morphology, are one of the principal cells implicated in the arthritic conditions in RA.[4] In RA, SFCs markedly increase in number and display transformed phenotypes, and the activation of various protooncogenes including *myc*, *ras*, and *fos* is involved in the abnormal growth rate and transcriptional activity of the cells.[5] Bone erosion usually begins at the interface of the cartilage and the proliferating synovium, and bone-resorbing osteoclasts can be observed at the erosive synovium/bone interfaces in RA joints. Accumulating evidence has revealed that osteoclasts, primary cells responsible for bone resorption, are involved in the bone destruction in RA, and recent progress in the molecular biology and biochemistry has elucidated the molecular mechanism of the osteoclast differentiation and activation. In contrast to such catabolic

S. Tanaka (✉)
Department of Orthopaedic Surgery, Faculty of Medicine,
The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
Tel. +81-3-3815-5411 (ext. 33376); Fax +81-3-3818-4082
e-mail: tanakas-ort@h.u-tokyo.ac.jp

actions, recent studies have revealed that synovial cells have anabolic effects on the joint homeostasis, and differentiate into osteoblasts or chondrocytes under proper conditions.

In this review, I would like to introduce the role of osteoclasts in the joint destruction in RA, and the critical involvement of SFCs on the osteoclast differentiation in RA. Adenovirus vectors can efficiently transduce osteoclasts and SFCs both in vitro and in vivo. By modulating intracellular signaling pathways in these types of cells using adenovirus vectors, we could regulate the joint destruction in the experimental animal models of arthritis. We also successfully induced chondrogenic differentiation of SFCs both in vitro and in vivo by stimulating transforming growth factor (TGF)-β/bone morphogenetic protein (BMP) signaling pathways.

Involvement of osteoclasts in bone destruction in RA

The cellular mechanism underlying the bone and cartilage destruction in RA is still unclear, but emerging evidence has revealed the essential role of osteoclasts. Bromley and Woolley[6] observed a number of acid phosphatase-positive multinucleated cells (chondroclasts and/or osteoclasts) in the erosive joint areas of RA patients. Gravallese et al.[7] found that multinucleated cells present on erosive bone surface and in the areas of the direct invasion of pannus into the subchondral bone. Abundant multinucleated giant cells were also observed at the bone-pannus interfaces of arthritic joints in collagen-induced arthritis rats.[8] Multinucleated cells were positive for unique markers of osteoclasts such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, and calcitonin receptors, satisfying the major criteria of mature osteoclasts.[7] Interestingly, some multinucleated cells and mononuclear cells apart from the bone surface were also TRAP-positive, suggesting the possible involvement of synovial tissues in the osteoclastogenesis in RA. To analyze the osteoclastogenic potentiality of RA synovial tissues, synovial cells were isolated from RA synovium at the time of knee replacement surgeries, and the cells were cultured in the presence of 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] and macrophage colony-stimulating factor (M-CSF). After 3 weeks of culture, there appeared many multinucleated giant cells, which were TRAP-positive, possessed abundant calcitonin receptors, and made resorption pits on dentine slices.[9] We also found that peripheral monocytes can differentiate into osteoclast-like cells when cocultured with SFCs. These results suggest that RA SFCs can support osteoclast differentiation from monocyte-macrophage lineage precursor cells.

Role of RANKL/RANK pathways in bone destruction in RA

Receptor activator of NF-κB ligand (RANKL) is a member of the TNF superfamily cytokines, which was originally identified as a membrane-bound survival factor for dendritic cells produced by activated T cells.[10,11] The expression of RANKL is also induced in osteoblasts or bone marrow stromal cells by various hormones or cytokines. In cooperation with M-CSF, RANKL stimulates osteoclast differentiation from hematopoietic precursor cells in vitro.[12] RANKL also acts on mature osteoclasts and promotes their bone-resorbing activity and survival. RANKL binds to its specific receptor RANK, the type I membrane receptor belonging to TNF receptor superfamily. RANK is expressed in wide range of cells including monocyte-macrophage lineage osteoclast precursor cells, mature osteoclasts and dendritic cells. Upon binding to its ligand RANKL, RANK recruits an adaptor molecule TNF receptor-associated factor (TRAF) 6, which subsequently activates downstream signaling pathways NF-κB, c-Jun N-terminus kinase (JNK), p38 mitogen-activated protein (MAP) kinase, and nuclear factor of activated T cells (NFAT) c1.[13] Another important actor in the RANKL/RANK pathway is osteoprotegerin (OPG), a soluble decoy receptor of RANKL which belongs to TNF receptor superfamily.[14,15] Osteoprotegerin specifically binds to RANKL and inhibits RANKL activity by competitively preventing its binding to RANK.

The essential role of RANKL/RANK signaling pathways in osteoclast development was further established by a series of gene knockout mice.[16] The targeted disruption of either RANKL or RANK induced osteopetrosis in mice, a pathological bone disease which is characterized by an increased bone mass due to a deficiency in osteoclast differentiation.[11,16] We and another group found that mice deficient in TRAF6 also showed osteopetrotic phenotypes.[17] In contrast, OPG-deficient animals demonstrated osteopenia due to the increased number and activity of osteoclasts.[18,19] These results clearly demonstrate the essential role of RANKL/RANK pathways in osteoclast development and activation in vivo.

Not only is the RANKL/RANK pathway critical for normal bone development and growth, but also is implicated in the pathological bone resorption observed in RA (Fig. 1). We and other groups found a high level expression of RANKL in RA synovial tissues.[20,21] Enhanced expression of RANKL is observed in SFCs as well as in $CD4^+$ T lymphocytes in synovial tissues of collagen-induced arthritis rats as shown by in situ hybridization.[22] Expression of RANKL is increased by T-cell proinflammatory cytokine IL-17, and IL-17 enhanced RANKL expression and strongly upregulated the RANKL/OPG ratio in the synovium.[23] Osteoprotegerin treatment ameliorates the arthritic bone destruction in adjuvant arthritis rats[24] and TNF-α transgenic animals,[25] and the bone erosion in serum transfer-induced arthritis was markedly reduced in RANKL-deficient animals.[26] Recent studies also demonstrated that the systemic bone loss as well as the local bone erosion in TNF-α transgenic mice was reversed by OPG injection in combination with anti-TNF-α antibody therapy.[27,28] These studies indicate that RANKL produced by SFCs and/or activated T lymphocytes in RA synovial tissues plays an essential role in the osteoclast development and the joint destruction, and therefore, the RANKL/RANK pathway can be a good therapeutic target.

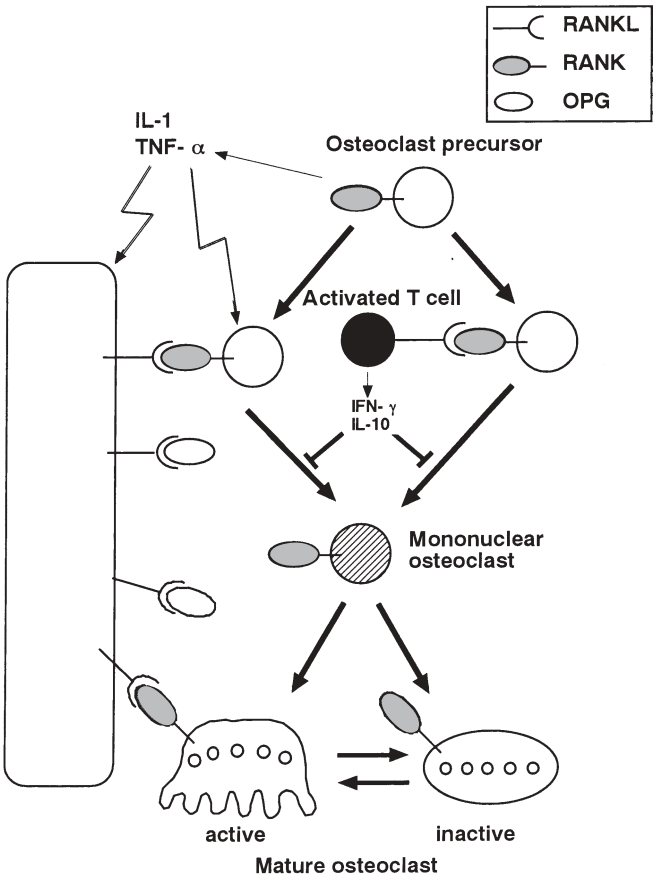

Fig. 1. Involvement of receptor activator of NF-κB ligand (*RANKL*)–receptor activator of NF-κB (*RANK*) pathways in osteoclast differentiation and bone destruction in RA. RANKL is highly expressed in synovial fibroblastic cells (SFCs) and activated T cells, and binds to its specific receptor RANK, which is expressed in monocyte-macrophage lineage osteoclast precursor cells. The interaction between RANKL and RANK is blocked by osteoprotegerin (*OPG*), a physiological inhibitor of RANKL. *IL*, interleukin; *TNF*, tumor necrosis factor; *IFN*, interferon

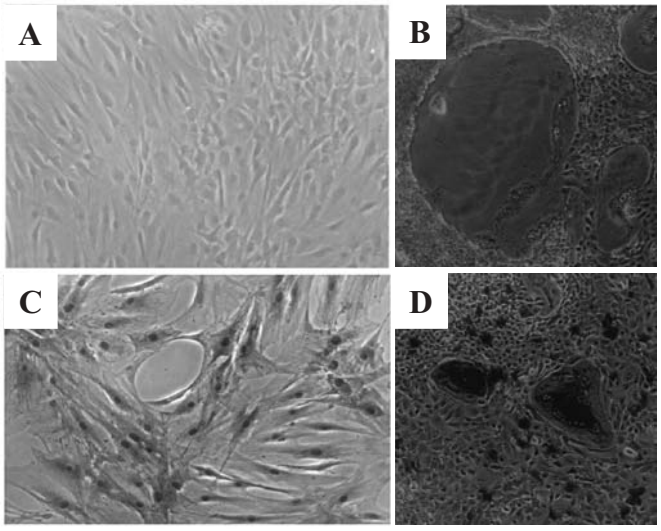

Fig. 2A–D. Effective gene transduction into SFCs and mature osteoclasts by adenovirus vectors. Human SFCs obtained from synovial tissues of rheumatoid arthritis patients (**A** and **C**) or osteoclast-like cells from giant cell tumors (**B** and **D**) were infected with the control virus (**A** and **B**) or LacZ virus (**C** and **D**), and stained for β-galactosidase activity 1 day after infection. Both SFCs and osteoclasts infected with LacZ virus were positively stained, indicating an efficient gene transduction

Efficiency of adenovirus vectors in transducing SFCs and osteoclasts

As mentioned above, osteoclasts play pivotal roles in the bone and joint pathology in RA, and SFCs support osteoclast differentiation and activation by producing RANKL. Therefore, pharmacological agents targeting these cells can be potent therapeutic candidates for the treatment of RA. One alternative is gene therapy, where genes or cDNAs are directly transferred to target cells. In preclinical studies, ex vivo and in vivo gene transfer methods have been used successfully to reduce the joint destruction in experimental arthritis, and the first clinical trial, in which the IL-1 receptor antagonist gene was delivered to synoviocytes ex vivo, was started in 1996 in the United States.[29,30] The proper selection of the target cells and target genes has been a continuous matter of interest for successful gene therapies. The target cells for the RA gene therapy include SFCs and osteoclasts, and we previously reported that adenovirus vectors efficiently transduce foreign genes into these cells both in vitro and in vivo.[31,32] As shown in Fig. 2, recombinant adenovirus vectors carrying the *lacZ* gene can infect human SFCs and osteoclast-like cells obtained from giant cell tumors.[31,32] At a multiplicity of infection of 100, almost 100% of SFCs and more than 85% of osteoclast-like cells were positively stained by β-galactosidase (β-gal) activity with no apparent morphological changes or cellular toxicity. When injected into knee joints of adjuvant arthritis rats, synovial lining cells and osteoclasts present on bone surface were positively stained for β-gal activity (Fig. 3).[32] These results suggest that the adenovirus vector system is suitable for gene therapies targeting SFCs and osteoclasts. As for target molecules, we focused on the intracellular signaling pathways which are important for both SFCs and osteoclasts, i.e., c-Src pathways and Ras/ERK pathways.

Adenovirus vector-mediated regulation of c-Src pathways in SFCs and osteoclasts

c-Src was first identified as the normal cellular counterpart of the transforming protein encoded by Rous sarcoma virus, v-Src.[33] The protooncogene product c-Src is a 60 kDa protein and belongs to non-receptor-type tyrosine kinase family, i.e., Src family tyrosine kinase family. The c-*src* protooncogene is highly conserved throughout evolution and widely expressed. It is known that c-Src and the other members of the Src family, which share highly conserved sequences both within and outside the kinase catalytic domain, play important roles in signal transduction mecha-

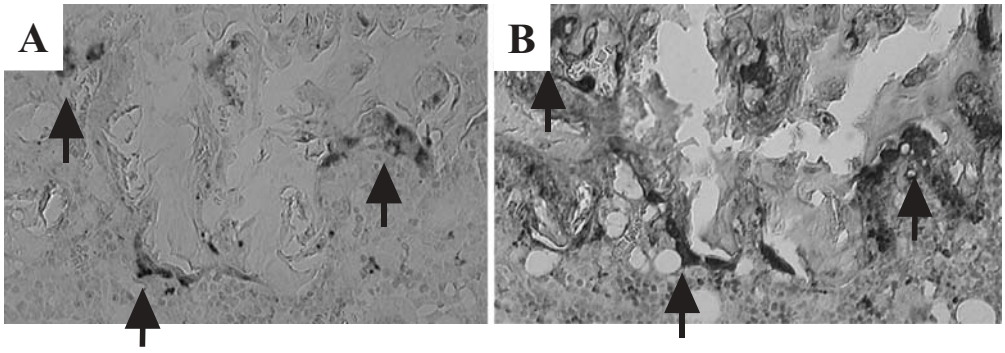

Fig. 3A,B. Adenovirus-mediated gene transduction in osteoclasts in vivo.[32] LacZ virus was injected into the inflammatory ankle joint of an adjuvant arthritis rat, and the expression *lacZ* gene in osteoclasts was determined in the serial sections by enzyme histochemistry of β-galactosidase (**A**) and tartrate-resistant acid phosphatase (TRAP) (**B**) after 1 week of the viral injection. Most of the TRAP-positive osteoclasts were positively stained for β-galactosidase activity

nisms that contribute to the regulation of cell growth and development.[33] The physiological role of the c-*src* gene had not been clarified until Soriano et al. successfully performed the targeted disruption of the gene by homologous recombination in mouse embryos in 1991.[34] To everyone's surprise, the mice showed striking skeletal abnormalities with a phenotype of osteopetrosis. In vitro osteoclast formation experiments and in vivo bone marrow transplantation studies have revealed that osteoclast differentiation was not impaired, but that bone-resorbing activity of mature osteoclasts was much reduced in c-*src* knockout (KO) mice.[35,36] The morphological feature of the KO osteoclasts was their disorganized ruffled border structure.[36] The ruffled border is the apical membrane of the osteoclast, which is extensively folded due to the intense vesicular traffic associated with proton and lysosomal enzyme secretion. c-Src is highly expressed in osteoclasts, and highly concentrated on ruffled border membranes and intracellular membranes.[37,38] The fact that no other abnormalities in c-*src* KO mice were found outside the skeletal tissues leads us to consider that c-Src can be an ideal therapeutic target for suppressing pathological bone resorption by inhibiting osteoclast function without affecting other tissues or cells.

The tyrosine kinase activity of c-Src is strictly regulated by phosphorylation and dephosphorylation of the tyrosine residue located close to the C-terminus, which corresponds to tyrosine 527 (Tyr527) in chicken c-Src.[39] Dephosphorylation of this residue causes a 10- to 20-fold increase in the kinase activity of c-Src. C-terminus Src family kinase (Csk) is a cytoplasmic tyrosine kinase which specifically phosphorylates Tyr527 of c-Src, thereby negatively regulate its kinase activity.[40] To regulate c-Src kinase activity in SFCs and osteoclasts, we constructed adenovirus vectors encoding *csk* gene (Csk virus). Csk virus efficiently infected SFCs and osteoclasts, and dose-dependently inhibited the kinase activity of c-Src in these cells.[41] Adenovirus vector-mediated Csk overexpression in RA SFCs suppressed the proliferation of the cells, and reduced their IL-6 production.[32] Csk virus also induced dramatic cytoskeletal disorganization in osteoclasts, and strongly inhibited pit formation on dentine slices.[41]

Important role of Ras/ERK pathways in SFC activation and osteoclast survival

The other signaling pathway we focused on as a therapeutic target is the Ras/ERK pathway. Small GTPase Ras, the protein product of proto-oncogene *ra*s, is ubiquitously found in eukaryotic organisms.[42] Ras is known to function as a downstream effector of cell surface receptor tyrosine kinases (RTKs) and leads to the activation of ERK pathways, which in turn regulates the activities of nuclear transcription factors and gene transcriptions. In human cancer cells, oncogenic mutations of Ras protein are frequently observed and contribute to the malignant growth properties of the cells. In RA and animal models of arthritis, synovial cells with large pale nuclei, prominent nucleoli and abundant cytoplasm are found adjacent to the affected cartilage and bone of the joint, and these cells in culture have a tendency to grow in disorganized monolayers, proliferate in an anchorage-independent manner, lack contact inhibition, and form microfoci, exhibiting a morphologically transformed appearance.[4] Although the expression of Ras and its oncogenic mutations were reported in RA synovial cells, the precise role of Ras in RA pathology remains unclear.[43] To analyze the role of Ras and its downstream signaling in osteoclasts as well as in SFCs, we constructed a replication-deficient adenovirus vector carrying the dominant negative mutant of *ras* gene (Ras^{DN}). In SFCs, adenovirus-mediated overexpression of Ras^{DN} dramatically decreased the proliferation rate of the cells. Interleukin-1-induced upregulation of IL-6 production was also decreased by the viral infection, which was supposedly mediated by the downregulation of IL-1-induced ERK activation.[44]

In addition, the life span of osteoclasts was markedly decreased by the adenovirus, while activating Ras/ERK pathways by constitutively active mutant of ERK expression prolonged the survival of osteoclasts.[45] These results suggest that Ras/ERK pathways are critically involved in SFC activation and osteoclast survival.

Amelioration of arthritic bone destruction by adenovirus vector-induced gene expression

The efficient in vivo gene delivery to synovial cells by local administration of adenovirus vectors has been well established.[32,46] In addition, TRAP-positive osteoclasts along the erosive bone surface demonstrated strong β-gal staining as shown in the serial tissue sections, indicating that intra-articular injection of adenovirus vectors can transduce osteoclasts in vivo (Fig. 3). The effect of Csk and Ras^{DN} adenovirus administration into inflammatory ankle joints of adjuvant arthritis rats was investigated.[32,44] Not only was the bone destruction by osteoclasts suppressed by Csk or Ras^{DN} virus injection, but also the synovial inflammatory reaction detected by arthritis score or paw swelling was reduced (Fig. 4). These results lead us to conclude that regulating c-Src and/or Ras/ERK pathways in SFCs and osteoclasts can be a novel therapeutic approach to treat RA joint destruction (Fig. 5).

Stimulating chondrogenic differentiation of SFCs

In contrast to such catabolic actions, recent studies have revealed that SFCs have anabolic effects, leading to the bone and cartilage production. Hunziker and Rosenberg[47] reported that synovial cells can migrate into partial-thickness articular cartilage defects, where they proliferate and subsequently deposit a scar-like tissue. Nishimura et al.[48] demonstrated SFCs to show chondrogenic differentiation after being cultured in the presence of TGF-β, and De Bari et al.[49] recently demonstrated that multipotent mesenchymal stem cells were isolated from human synovial tissues, which differentiated into chondrocytes as well as osteoblasts, adipocytes, and myotubes under proper culture conditions. These observations lead us to speculate that synovial tissues contain multipotent cells with osteogenic and/or chondrogenic potential that can be involved in the repair process of destroyed joints and therefore might provide a good source for engineering the bone and cartilage.

There is accumulating evidence that TGF-β superfamily cytokines play an essential role in bone and cartilage development. We analyzed the role of TGF-β/BMP signaling on chondrogenic differentiation of human SFCs, and found that the introduction of an activated mutant of ALK3 (con-

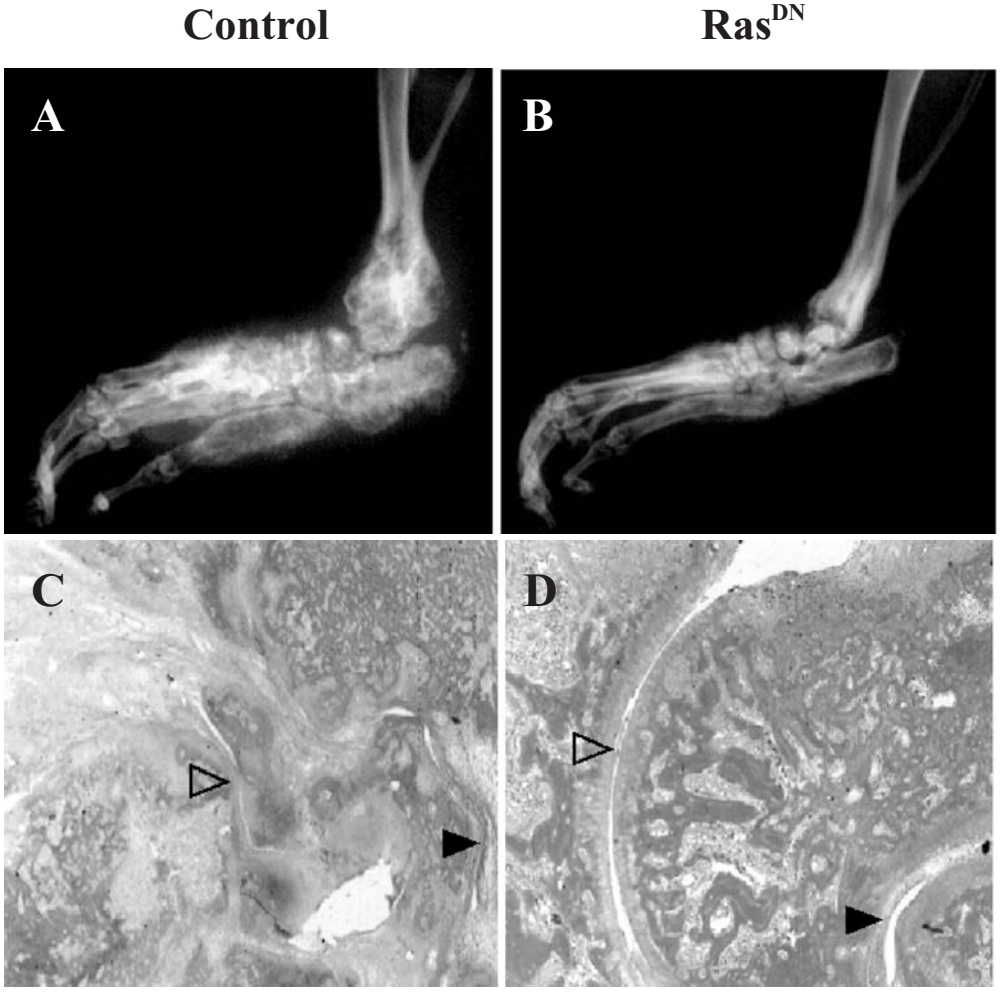

Fig. 4A–D. Therapeutic effects of dominant negative Ras (*Ras^{DN}*) adenovirus injection on rat adjuvant arthritis. **A,B** The radiological findings of the ankle of LacZ virus- (**A**) and Ras^{DN} virus- (**B**) injected rats. Severe joint destruction could be seen in LacZ virus-injected rats, while Ras^{DN} virus-injected ankle joints show minimal destructive changes. **C** Pathohistological findings of the LacZ virus-injected ankles show synovial hyperplasia and destructive change of articular cartilage and bone. *Open arrowhead* and *closed arrowhead* indicate talo-tibial and talo-calcaneal joint, respectively. **D** Pathohistological findings of the Ras^{DN} virus-injected ankle. Synovial hyperplasia with invasion into subchondral bone and the destruction of bone and cartilage were markedly suppressed. **C**,**D**: H&E staining

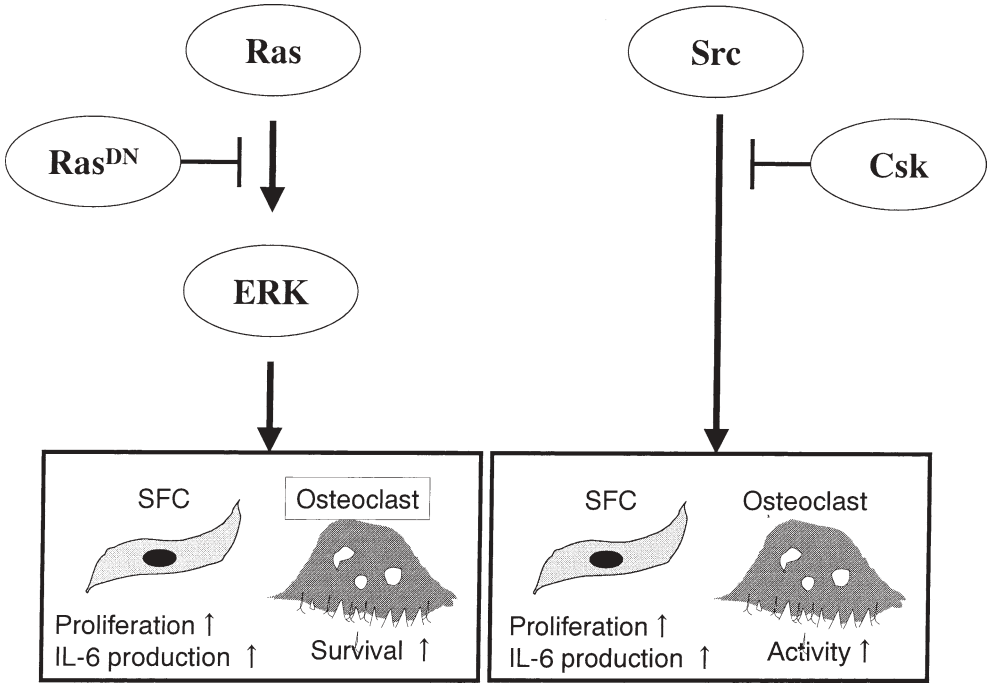

Fig. 5. c-Src pathways and Ras/extracellular-regulating kinase (*ERK*) pathways are good therapeutic targets for the joint destruction in rheumatoid arthritis. Suppression of c-Src pathways by adenovirus vector-mediated C-terminal Src family kinase (*Csk*) overexpression inhibits the proliferation and interleukin-6 (*IL-6*) production of synovial fibroblastic cells (*SFC*) and osteoclast activity. Ras/ERK pathways are involved in SFC activation and osteoclast survival, and suppressed by dominant negative Ras (Ras^{DN}) induction

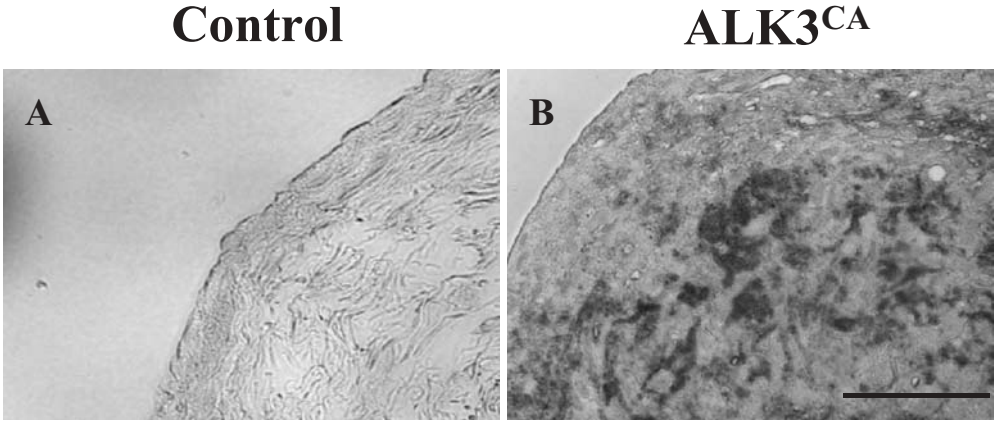

Fig. 6A,B. Constitutively active activin receptor-like kinase ($ALK3^{CA}$)-transduced SFs form cartilage matrix in vivo. Three weeks after transplantation to nude mice, the pellets were recovered and immunostained with anti-type II collagen antibody. Clear positive stainings were observed in $ALK3^{CA}$-expressing cultures (**B**) in contrast to LacZ virus-infected cultures (**A**). *Bar* 100 μm

stitutively active activin receptor–like kinase 3 [$ALK3^{CA}$]), also known as BMP type IA receptor, by adenovirus vectors induced chondrocyte-specific marker expression in the cells.[50] Overexpression of $ALK3^{CA}$ induced type II collagen and aggrecan expression in SFCs, and when transplanted into nude mice, the transplanted SFC pellets expressing $ALK3^{CA}$ were positively stained for type II collagen immunostaining, indicating the cartilaginous differentiation of the cultures in vivo (Fig. 6). ALK3 signaling involves two different downstream cascades, the Smad pathway and the p38 MAP kinase pathway. We used a combination of adenoviral gene delivery and chemical inhibition to analyze the role of these two signaling cascades in inducing differentiation of SFCs toward a chondrocyte phenotype and found that both pathways are essential for chondrogenic differentiation.[50] Interestingly, activation of p38 pathways alone induced markers of terminal chondrocyte differentiation, type X collagen expression, and mineralization, which was suppressed by Smad1 coexpression.[50] Because type X collagen expression and mineralization are observed in degenerated cartilage but not in intact articular cartilage, our findings suggest an important role for p38 signal transduction pathways in chondrocytes and SFs, leading to degenerative joint disorders, and the potential usefulness of p38 modifiers in the treatment of RA. These results suggest that although p38 activation is necessary for chondrogenic differentiation of SFs, sustained activation of the pathway promotes the terminal differentiation of the cells (Fig. 7). Consistent with our results, Zhen et al.[51] reported that parathyroid hormone inhibits type X collagen expression in hypertrophic chondrocytes by suppressing p38 pathways. Smad pathways are not only required for chondrogenic

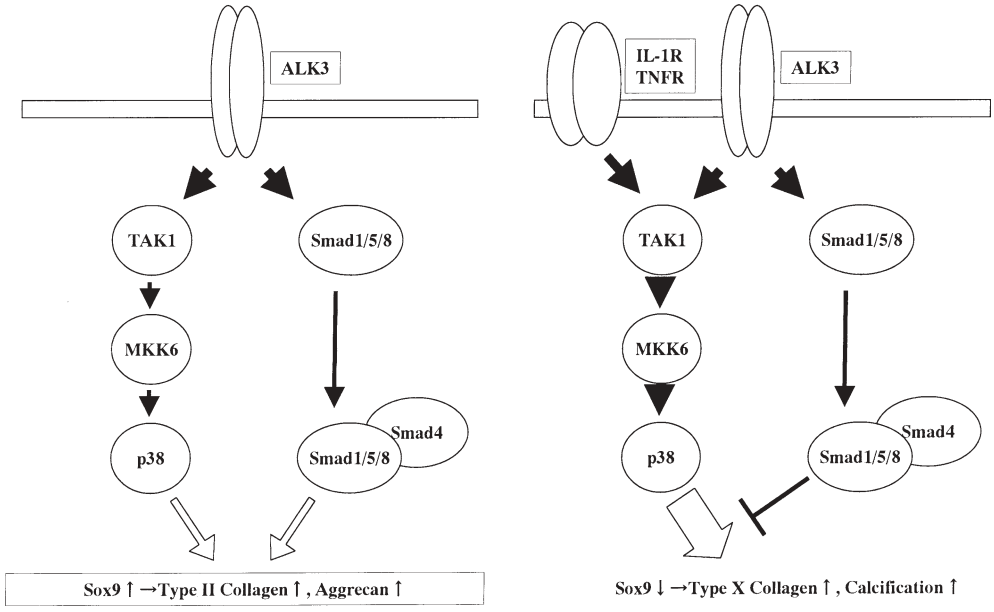

Fig. 7. The role of the Smad pathway and p38 mitogen-activated protein kinase pathway on chondrogenic differentiation of SFCs. Although both pathways are necessary for chondrocyte-specific marker expression in the cells (*left panel*), overactivation of p38 pathways alone lead to the terminal chondrocytic differentiation of the cells, leading to the articular cartilage degeneration. The proper balance between these two pathways is required for maintaining the articular cartilage integrity. *IL*, interleukin; *TNFR*, tumor necrosis factor receptor; *ALK*, activin receptor-like kinase

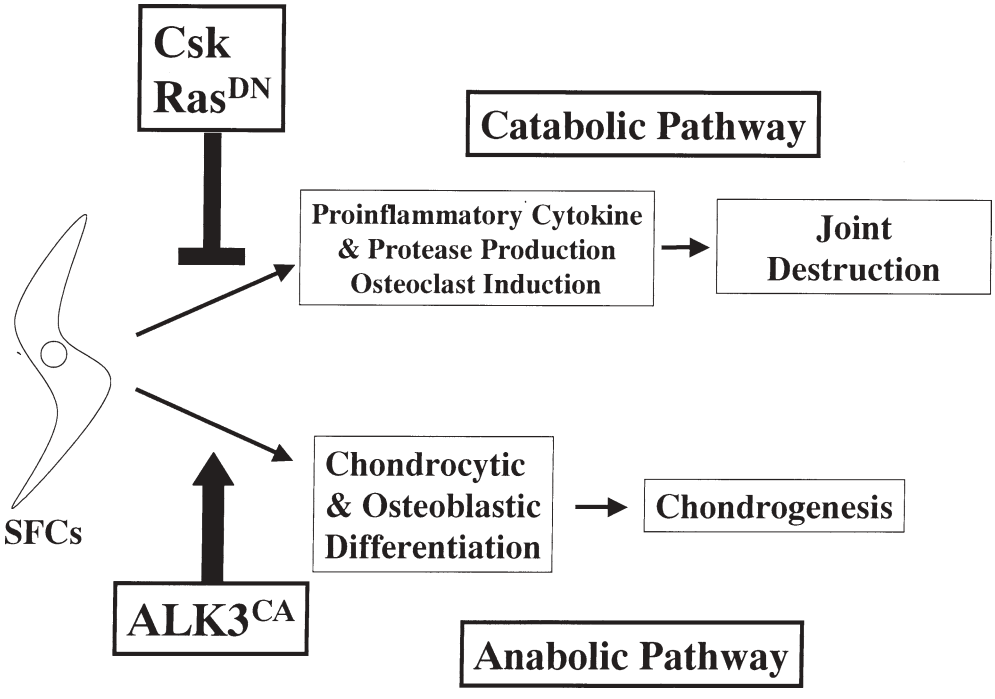

Fig. 8. Schematic representation of the therapeutic strategies targeting synovial fibroblastic cells (*SFCs*). Suppressing Src pathways by C-terminal Src family kinase (*Csk*) expression or Ras/ERK pathways by dominant negative Ras (Ras^{DN}) expression suppresses the catabolic pathways of the cells which lead to the bone and joint destruction in RA, while stimulating ALK3 pathways activates anabolic pathways leading to chondrogenic differentiation of the cells. $ALK3^{CA}$, constitutively active activin receptor-like kinase

differentiation of SFs, but also regulate the stage of differentiation of the cells and suppress their terminal differentiation process. It should be noted that the proinflammatory cytokines IL-1 and TNF-α, which are known to have catabolic effects on joint integrity, induced p38 activation in SFs.[52] These cytokines may stimulate terminal chondrogenic differentiation of SFs which are involved in the repair process, and lead to the articular cartilage degradation. Based on our observation, we would like to propose that SFs are an excellent source from which to obtain chondroprogenitors, which can be differentiated into chondrocytes via ALK3 activation, and that stimulating Smad pathways and controlling p38 activation to the proper level can be a good therapeutic strategy for maintaining the healthy joint homeostasis and treating degenerative joint disorders.

Concluding remarks

The ultimate goal of the treatment of RA is to preserve the daily activity of the patients by preventing bone and joint destruction. Recent studies have revealed that osteoclasts are involved in the pathogenesis of the bone and joint destruction in RA, and SFCs are critically involved in the differentiation and activation of osteoclasts by producing various catabolic factors including TNF-α, IL-1, and RANKL, which makes osteoclasts and SFCs good therapeutic targets for bone and joint destruction in RA. We demonstrated that suppressing Src pathways by introducing Csk and/or Ras/ERK pathways by the Ras^{DN} adenovirus in osteoclasts reduces bone resorption both in vitro or in vivo by suppressing osteoclast activity or survival (Fig. 5). Csk virus and Ras^{DN} virus also suppress catabolic actions of SFCs by inhibiting abnormal proliferation of the cells and their IL-6 production (Fig. 5). We also succeeded to stimulate chondrogenic differentiation of SFCs by introducing $ALK3^{CA}$, and the proper balance of the Smad pathway and the p38 MAP kinase pathway is critical downstream of ALK3. Based on these observations, I would like to propose that modulating intracellular signaling in osteoclasts and/or SFCs by adenovirus vectors can be good therapeutic approach for treating RA patients with bone and joint destruction (Fig. 8).

Of course, we have to realize the disadvantages as well as advantages of using adenovirus vectors as therapeutic reagents.[53] The disadvantages of the adenovirus vectors include the transient gene expression because they do not integrate the transgene into target cell chromosome, immunological reaction such as neutralizing antibody response and cytotoxic T-lymphocyte responses against the virus, and the dissemination of the vectors from the site of local injection.[54] The safety issue is particularly important regarding its clinical application, and in fact, the first case of fatality induced by the infusion of adenovirus vectors into hepatic artery was recently reported. Therefore, development of a new generation of adenovirus vectors or finding substitutes for gene therapy is absolutely necessary for the clinical application of the signaling molecule-targeting strategies.

Acknowledgments This work was in part supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and Health Science Research Grants from the Ministry of Health, Labour and Welfare, to S.T.

References

1. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.
2. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000;343:1594–602.
3. Sorsa T, Konttinen YT, Lindy O, Ritchlin C, Saari H, Suomalainen K, et al. Collagenase in synovitis of rheumatoid arthritis. Semin Arthritis Rheum 1992;22:44–53.
4. Firestein GS. Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Passive responders or transformed aggressors? Arthritis Rheum 1996;39:1781–90.
5. Muller-Ladner U, Kriegsmann J, Gay RE, Gay S. Oncogenes in rheumatoid arthritis. Rheum Dis Clin North Am 1995;21:675–90.
6. Bromley M, Woolley DE. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. Arthritis Rheum 1984;27:968–75.
7. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 1998;152:943–51.
8. Suzuki Y, Nishikaku F, Nakatuka M, Koga Y. Osteoclast-like cells in murine collagen induced arthritis. J Rheumatol 1998;25:1154–60.
9. Takayanagi H, Oda H, Yamamoto S, Kawaguchi H, Tanaka S, Nishikawa T, et al. A new mechanism of bone destruction in rheumatoid arthritis: synovial fibroblasts induce osteoclastogenesis. Biochem Biophys Res Commun 1997;240:279–86.
10. Wong BR, Josien R, Choi Y. TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. J Leukocyte Biol 1999;65:715–24.
11. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 1999;20:345–57.
12. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998;95:3597–602.
13. Tanaka S, Nakamura I, Inoue J, Oda H, Nakamura K. Signal transduction pathways regulating osteoclast differentiation and function. J Bone Miner Metab 2003;21:123–33.
14. Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, et al. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 1997;234:137–42.
15. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
16. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337–42.
17. Inoue J, Ishida T, Tsukamoto N, Kobayashi N, Naito A, Azuma S, et al. Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. Exp Cell Res 2000;254:14–24.
18. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, et al. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260–8.
19. Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, et al. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun 1998;247:610–5.
20. Takayanagi H, Iizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, et al. Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 2000;43:259–69.
21. Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E, et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum 2000;43:250–8.
22. Romas E, Bakharevski O, Hards DK, Kartsogiannis V, Quinn JM, Ryan PF, et al. Expression of osteoclast differentiation factor at sites of bone erosion in collagen-induced arthritis. Arthritis Rheum 2000;43:821–6.
23. Lubberts E, van den Bersselaar L, Oppers-Walgreen B, Schwarzenberger P, Coenen-de Roo CJ, Kolls JK, et al. IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. J Immunol 2003;170:2655–62.
24. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402:304–9.

25. Redlich K, Hayer S, Maier A, Dunstan CR, Tohidast-Akrad M, Lang S, et al. Tumor necrosis factor alpha-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. Arthritis Rheum 2002;46:785–92.
26. Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001;159:1689–99.
27. Redlich K, Gortz B, Hayer S, Zwerina J, Doerr N, Kostenuik P, et al. Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. Am J Pathol 2004;164:543–55.
28. Zwerina J, Hayer S, Tohidast-Akrad M, Bergmeister H, Redlich K, Feige U, et al. Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. Arthritis Rheum 2004;50:277–90.
29. Evans CH, Ghivizzani SC, Herndon JH, Wasko MC, Reinecke J, Wehling P, et al. Clinical trials in the gene therapy of arthritis. Clin Orthop 2000;S300–7.
30. Evans CH, Gouze JN, Gouze E, Robbins PD, Ghivizzani SC. Osteoarthritis gene therapy. Gene Ther 2004;11:379–89.
31. Tanaka S, Takahashi T, Takayanagi H, Miyazaki T, Oda H, Nakamura K, et al. Modulation of osteoclast function by adenovirus vector-induced epidermal growth factor receptor. J Bone Miner Res 1998;13:1714–20.
32. Takayanagi H, Juji T, Miyazaki T, Iizuka H, Takahashi T, Isshiki M, et al. Suppression of arthritic bone destruction by adenovirus-mediated csk gene transfer to synoviocytes and osteoclasts. J Clin Invest 1999;104:137–46.
33. Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 1997;13:513–609.
34. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64:693–702.
35. Lowe C, Yoneda T, Boyce BF, Chen H, Mundy GR, Soriano P. Osteopetrosis in Src-deficient mice is due to an autonomous defect of osteoclasts. Proc Natl Acad Sci USA 1993;90:4485–9.
36. Boyce BF, Yoneda T, Lowe C, Soriano P, Mundy GR. Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice. J Clin Invest 1992;90:1622–7.
37. Horne WC, Neff L, Chatterjee D, Lomri A, Levy JB, Baron R. Osteoclasts express high levels of pp60c-src in association with intracellular membranes. J Cell Biol 1992;119:1003–13.
38. Tanaka S, Takahashi N, Udagawa N, Sasaki T, Fukui Y, Kurokawa T, et al. Osteoclasts express high levels of p60c-src, preferentially on ruffled border membranes. FEBS Lett 1992;313:85–9.
39. Brown MT, Cooper JA. Regulation, substrates and functions of src. Biochim Biophys Acta 1996;1287:121–49.
40. Okada M, Nada S, Yamanashi Y, Yamamoto T, Nakagawa H. CSK: a protein-tyrosine kinase involved in regulation of src family kinases. J Biol Chem 1991;266:24249–52.
41. Miyazaki T, Takayanagi H, Isshiki M, Takahashi T, Okada M, Fukui Y, et al. In vitro and in vivo suppression of osteoclast function by adenovirus vector-induced csk gene. J Bone Miner Res 2000;15:41–51.
42. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: conserved structure and molecular mechanism. Nature 1991;349:117–27.
43. Gay S, Gay RE. Cellular basis and oncogene expression of rheumatoid joint destruction. Rheumatol Int 1989;9:105–13.
44. Yamamoto A, Fukuda A, Seto H, Miyazaki T, Kadono Y, Sawada Y, et al. Suppression of arthritic bone destruction by adenovirus-mediated dominant-negative Ras gene transfer to synoviocytes and osteoclasts. Arthritis Rheum 2003;48:2682–92.
45. Miyazaki T, Katagiri H, Kanegae Y, Takayanagi H, Sawada Y, Yamamoto A, et al. Reciprocal role of ERK and NF-kappaB pathways in survival and activation of osteoclasts. J Cell Biol 2000;148:333–42.
46. Nita I, Ghivizzani SC, Galea-Lauri J, Bandara G, Georgescu HI, Robbins PD, et al. Direct gene delivery to synovium. An evaluation of potential vectors in vitro and in vivo. Arthritis Rheum 1996;39:820–8.
47. Hunziker EB, Rosenberg LC. Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. J Bone Joint Surg Am 1996;78:721–33.
48. Nishimura K, Solchaga LA, Caplan AI, Yoo JU, Goldberg VM, Johnstone B. Chondroprogenitor cells of synovial tissue. Arthritis Rheum 1999;42:2631–7.
49. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928–42.
50. Seto H, Kamekura S, Miura T, Yamamoto A, Chikuda H, Ogata T, et al. Distinct roles of Smad pathways and p38 pathways in cartilage-specific gene expression in synovial fibroblasts. J Clin Invest 2004;113:718–26.
51. Zhen X, Wei L, Wu Q, Zhang Y, Chen Q. Mitogen-activated protein kinase p38 mediates regulation of chondrocyte differentiation by parathyroid hormone. J Biol Chem 2001;276:4879–85.
52. Schett G, Tohidast-Akrad M, Smolen JS, Schmid BJ, Steiner CW, Bitzan P, et al. Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum 2000;43:2501–12.
53. Mountain A. Gene therapy: the first decade. Trends Biotechnol 2000;18:119–28.
54. Benihoud K, Yeh P, Perricaudet M. Adenovirus vectors for gene delivery. Curr Opin Biotechnol 1999;10:440–7.