Ras family GTPase signaling contributions to inflammation and joint destruction in rheumatoid arthritis

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chapter 1

Introduction
H-Ras is de homoloog van RasGTPase welke de grootste bijdrage levert aan de beschadiging van gewrichten in RA
Introduction

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is one of the most common chronic immune-mediated inflammatory diseases in the western world, affecting up to 1% of the population, and having an increased incidence among women (1,2). RA is predominantly characterized by inflammation of synovial tissue lining in the joints (3). Progressive infiltration of the involved joints by inflammatory white blood cells and hyperplasia of stromal fibroblast-like synoviocytes in the intimal lining layer leads to pain, destruction of cartilage and bone tissue, and eventual patient disability (4). The etiology of RA is still unknown, but recent advances in our knowledge of cytokines and cell populations important to pathology in RA have led to the introduction of new biological therapeutic compounds which can alleviate inflammation and slow joint destruction. These compounds, such as tumor necrosis factor a (TNFa) blockers, anti-interleukin (IL)-6 receptor antibody treatment, B cell depletion, and inhibitors of T cell costimulatory molecules, provide marked clinical benefit, but disease in a significant number of patients is still refractory to these therapies, and current treatment rarely leads to disease remission (5). Therefore, the continued development of new therapeutic compounds, based on a better understanding of the pathobiology of RA, is needed. In particular, identification of key intracellular signaling pathways needed to sustain cellular activation and survival in RA synovial tissue may provide a basis for the development of new therapeutic strategies (6).

Fibroblast-like Synoviocytes

Joint destruction in RA results from invasion of cartilage and bone by the pannus, a hyperplastic tissue mass composed of inflammatory immune cells (primarily macrophages, T lymphocytes, and plasma cells, as well as B lymphocytes, natural killer cells, mast cells, neutrophils and dendritic cells) and stromal fibroblast-like synoviocytes (FLS) (4). In normal healthy tissue, FLS form a lining along the synovial cavity two to three cell layers deep. FLS are thought to be of mesenchymal origin and secrete factors which nourish and lubricate the joint (7,8). However, in RA, as well as other forms of arthritis, activated FLS accumulate along the intimal lining layer, secreting chemokines, cytokines and matrix metalloproteinases (MMPs) which perpetuate inflammation and directly or indirectly promoting cartilage degradation and bone erosion (8,9). It is uncertain if this accumulation of FLS arises from enhanced differentiation of precursor cells, defects in cellular apoptosis, or increased FLS proliferation, and these possibilities are a focus of extensive study that remain to be resolved. However, recent studies have indicated that RA FLS proliferate quite slowly in vivo (10).
Investigators conducting early histological evaluations were struck by the gross similarities between RA synovial tissue and solid tumors found in cancer patients (11). It was also observed that RA FLS maintained in culture in vitro displayed behavioral properties often associated with cellular transformation by oncogenes (12). Examples include the enhanced proliferative capacity of RA FLS compared to FLS obtained from healthy tissue or patients with other forms of arthritis, the ability of RA FLS to grow in an anchorage-independent manner in vitro, their resistance to cell–cell contact inhibition in regard to growth, and findings that RA FLS constitutively secrete autocrines and MMPs. Remarkably, activated FLS can migrate to unaffected joints, initiating inflammation and cartilage destruction at distal locations, a phenomenon markedly analogous to tumor cell metastasis in cancer (13). It is clear that an inflammatory phenotype is imprinted upon RA FLS, as gene expression profiles of RA patient synovial tissue and FLS cultured from ex vivo from the same patients are highly similar (14). Additionally, invasive properties of RA FLS in vitro highly correlate with the rate of joint destruction occurring in the patients from which they were obtained (15,16). However, the molecular mechanisms contributing to this "semi–transformed" phenotype of RA FLS are poorly understood.

One possible mechanism which might contribute to the "semi–transformed" phenotype of RA FLS is that initial inflammatory events in the synovium generate somatic mutations in proto–oncogenes or tumor suppressors (17). Indeed, inactivating mutations in the tumor suppressor p53, consistent with those caused by oxidative stress, can be found in oligoclonal populations of FLS in a subset of RA patients (18,19). Attempts to identify mutations in other gene products commonly involved in cellular transformation, such as Ras proto–oncogenes and the tumor suppressor phosphatase and tensin homologue deleted on chromosome ten (PTEN), have not been successful (20,21). Another contributing factor to the pathogenic behavior of RA FLS may be changes in the epigenetic regulation of proto–oncogene and tumor suppressor expression, as well as gene products such as cytokines which contribute to disease in RA (22). DNA methylation is an epigenetic mechanism which can repress gene transcription by either preventing recruitment of transcription factors to gene promoters, or inducing recruitment of transcriptional co-repressors. In RA synovial tissue, global hypo–methylation of genomic DNA is observed, compared to the synovial tissue of non–inflammatory osteoarthritis (OA) patients. This global hypo–methylation of DNA is preserved in RA FLS, and exposure of normal fibroblasts to 5–azacytidine, a DNA hypo–methylating drug, can confer stable phenotypic and gene expression patterns resembling RA FLS (23). However, this same study observed that 5–azacytidine could further enhance expression of adhesion proteins, growth factor receptors and MMP production in RA FLS. Thus, the persistent activation of intracellular signal transduction pathways promoting RA FLS activation and survival in vivo is in great part a result of constant exposure of the cells to a complex inflammatory cytokine and cell–cell milieu (6,9).
Ras Gtpases

The Ras GTPase super-family is comprised of several hundred related intracellular signaling proteins which are regulated and function through evolutionarily conserved mechanisms. Members of this super-family are small molecular weight guanine nucleotide-binding proteins, named after the prototypical family of Ras homologues (24). Ras GTPase signaling is reversibly regulated by the binding of GDP and GTP. When bound to GDP, Ras GTPases are inactive, but cellular stimuli can activate guanine nucleotide exchange factors (GEFs), which displace GDP from specific GTPases. Freed from GDP, the GTPase quickly binds GTP, which is found in a molar excess to GDP within the cell. Binding of GTP to Ras proteins induces a conformational change, allowing GTPases to associate with and activate multiple downstream signaling proteins (25). Ras super-family members possess intrinsic GTPase activity, which hydrolyzes bound GTP to GDP, and allows the protein to return to its inactive state. However, this intrinsic GTPase activity is too slow to allow efficient temporal regulation of signaling, and is instead catalyzed by specific GTPase-activating proteins (GAPs), which negatively regulate GTPase function (25). Point mutations which abrogate the ability of GAPs to catalyze inactivation of GTPases lead to the potent activation of Ras family proteins, rendering them oncogenic (25). Indeed, activating mutations of Ras proteins are found in the majority of solid human tumors (26).

The closely related Ras family GTPase homologues (H-, K-, and N-Ras) are expressed throughout mammalian tissue, and play important roles in coupling extra-cellular stimuli to multiple downstream signaling pathways. Genetic studies have indicated that at least during development, Ras homologues have a high degree of functional redundancy. No obvious developmental problems or readily evident phenotypes are observed in mice lacking H-Ras, N-Ras or the K-Ras4A splice variant, alone or in combination. Genetic deletion of both splice variants of K-Ras, in contrast, results in embryonic lethality (27,28). The ability of many of the Ras homologues to functionally compensate for each other during development likely arises from their highly shared sequence identity, especially in the effector domain, which directly couples GTPases to downstream signaling proteins. Mitogen-activated protein (MAP) serine/threonine kinase cascades, Ral GTPase signaling, and phosphatidylinositol 3-kinase (PI3 kinase) targets represent the best-characterized signaling pathways activated by Ras family GTPases, and signaling via each of these pathways contributes to Ras-mediated transformation (29).

Despite the functional redundancies observed for H-, K-, and N-Ras proteins during development, genetic and cell biology studies have provided strong evidence that each of the Ras homologues has distinct signaling properties. Specificity in Ras homolog signaling is conferred in part by differential subcellular localization of each homolog. Unique carboxy (C)-terminal sequences and differential post-translational modifications of the C-terminal peptide directly localize of Ras homologues to distinct cellular membrane compartments, and regulate the inclusion or exclusion of Ras homologues from lipid rafts (30). This in turn impacts upon the ability of each Ras protein to interact with distinct
GEFs and downstream effector proteins - H-Ras, K-Ras, and N-Ras proteins demonstrate hierarchal efficiencies in activating MAP kinase and PI3 kinase signaling pathways (31,32). However, this selective activation of distinct downstream signaling pathways by each Ras protein may be only cell-type specific, as another layer of complexity is added by the observation that immediate downstream targets of Ras proteins, for example Raf proteins in the MAP kinase cascade, discussed below, are often part of a family of related proteins, each of which is preferentially activated by distinct Ras proteins (33). Thus, in one cell type, only N-Ras may activate the MAP kinase cascade, while in another cell type, each Ras protein may activate this signaling pathway. Despite this high potential for functional redundancies, different gene expression profiles have been noted in H-Ras and N-Ras knockout mice, and N-Ras knockout mice demonstrate defects in their T cell compartment during viral infection (34,35).

Initial immunohistochemical studies indicated that Ras proteins were expressed in RA synovial tissues, but drew differing conclusions regarding whether Ras expression was elevated in RA synovial tissue compared to disease controls and healthy individuals (36,37). In these studies, antibodies lacking specificity to distinguish between Ras homologues were used, perhaps explaining discrepancies in the findings. However, numerous independent studies have provided evidence that Ras proteins play an important role in RA FLS activation, and contribute to pathology in animal models of RA. Farnesyl-transferase inhibitors, which prevent proper membrane localization of Ras family members and signaling components of G protein-coupled receptors decrease the incidence and severity of collagen-induced arthritis (CIA) in mice, accompanied by decreased synovial TNF α and IL-1β mRNA expression (38). Over-expression of a dominant-negative c-Raf kinase in RA FLS, which broadly binds to and inhibits Ras homologues and related Ras family members, suppresses growth factor-induced activation of the extracellular-regulated kinase (ERK) and c-jun N-terminal kinase (JNK) MAP kinases, interferes with FLS MMP production, and slows FLS proliferation and invasiveness in vivo (39). Presumably more selective interference with H-Ras signaling, via ectopic expression of dominant-negative H-Ras, suppresses IL-1β-induced ERK activation and IL-6 production in RA FLS, and suppresses joint destruction in experimental arthritis (40). Together these studies indicate a contributory role for Ras proteins in RA, but the comparative involvement of distinct Ras homologues in pathology has not been examined.

RAS Activation and Guanine Nucleotide Exchange Factors
Activation of Ras proteins is regulated by three major groups of Ras GEFs, the mammalian son-of-sevenless (mSos) proteins, Ras guanine nucleotide releasing proteins (RasGRPs) and Ras guanine nucleotide-releasing factors (RasGRFs) (41). mSos proteins are recruited to activated receptor tyrosine kinase or non-receptor tyrosine kinases via adaptor proteins such as Grb2, which bind to tyrosine-phosphorylated signaling complexes via their Src-homology 2 (SH2) domains (42). This localizes mSos near the plasma membrane, allowing interaction of mSos with membrane phospholipids via its pleck-
strin homology (PH) domain, and subsequent activation (43). RasGRPs are abundantly expressed in brain tissue and within the immune system, predominantly in lymphocytes (41). Unlike mSos, the four RasGRP proteins are regulated not by protein–protein binding interactions, but rather, are activated to varying degrees by intracellular calcium and/or binding to diacyl glycerol (DAG) or other phorbol esters (44–47). The molecular mechanisms by which RasGRFs are activated are still poorly understood, but could result from calcium-dependent activation of associated calmodulin protein, phosphorylation by protein kinase A (PKA), or phosphorylation by non-receptor tyrosine kinases (41). Additionally, protease-dependent cleavage may be an important mechanism for regulating RasGRF activation, at least in melanoma cancer cell lines (48,49).

Ras GEFs in general show little specificity in the activation of distinct Ras homologues. mSos proteins can catalyze GTP exchange on all Ras homologues (50,51). Curiously, RasGRPs appear to selectively target Ras homologues in response to phorbol ester stimulation, and the related Rap GTPases in response to calcium (45). RasGRF1 appears to have the highest selectivity in vivo, preferentially activating H–Ras but not K–Ras, N–Ras or R–Ras (51,52). An added layer of complexity in Ras GEF biology is that mSos and RasGRP proteins also contain catalytic domains which can activate Rho family Rac GTPases, which in turn stimulate downstream signaling pathways which overlap with those of Ras proteins (41). To date, no studies have examined the potential contributions of specific Ras GEFs in the maintenance of inflammation or promotion of joint destruction in RA.

Mitogen-Activated Protein (MAP) Kinases
MAP kinases, consisting of the p38 (α, β, γ and δ isoforms), ERK 1 and 2, and JNK 1–3, are activated by many extracellular stimuli, including growth factors and inflammatory cytokines (53,54). These enzymes play a central role in coupling receptor ligation to regulation of gene transcription, often by Ras and Ras super-family–dependent mechanisms. In general, MAP kinases are activated following the initiation of a serine/threonine kinase signaling cascade (54). Phosphorylation of MAP kinases by upstream MAP kinase kinase (MAPKK or MEK) is sufficient and required to activate MAP kinases. MEKs in turn are activated by phosphorylation by a MAPKK kinase (MAPKKK or MEKK). MEKKs are directly stimulated by active Ras and Rho family GTPases, as well as non-receptor kinases which are recruited to activated receptors (53). While p38, ERK, and JNK MAP kinases are thought to be involved primarily in inflammatory, mitogenic, and stress responses, respectively, most receptors activate each of the MAP kinase pathways simultaneously. In some cases, Ras–dependent activation of MAP kinases can be quite indirect, as typified by the ability of Ras to stimulate JNK proteins via Ras–dependent activation of the small GTPase Ral (55). Once activated, each MAP kinase can phosphorylate numerous cellular proteins, many of which are transcription factors (56). Well-characterized transcription factor targets of p38 are Elk-1, NF-κB, and ATF1. Targets of ERK include c-Fos, Elk-1, NF-AT, MEF2, STAT3 and myc. c-Jun, STAT3 and HSF-1 are amongst some of the transcription factor targets of JNK.
Each MAP kinase is expressed and detected in an activated, phosphorylated form in RA synovial tissue (57,58). This observation, combined with a number of *in vitro* and *in vivo* studies described below, and generated tremendous interest in the therapeutic potential of targeting these enzymes in RA (6,59,60).

**P38 MAP Kinase**
Phosphorylated p38 is readily observed in RA synovial tissue, and this MAP kinase, as well as ERK and JNK proteins, is activated following inflammatory stimulation of RA FLS (57). Activation of in RA FLS is largely dependent upon MEK3 and MEK6, although MEK3 appears to play a dominant role in murine CIA (61,62). MEKKs leading to p38 activation are generally not regulated by Ras GTPases, but rather Rho family GTPase (53). However, as mentioned above, Ras GEFs can stimulate Rho family proteins, and some evidence has emerged that Ras-dependent activation of Raf proteins (54). Numerous and increasingly specific p38 inhibitors have shown potent therapeutic properties in animal models of arthritis (63-65), and the potential of these compounds as therapeutic agents in the treatment of RA was enhanced by studies in human TNF$\alpha$ transgenic mice, a spontaneous arthritis model. In this model, arthritis development is associated with the selective activation of p38 and ERK, but only negligible JNK activation is observed (66). Importantly, pharmacological inhibition of p38, but not JNK1, blocks arthritis in this model (67,68). However, it is increasingly unclear if involvement of p38 in these animal models is relevant to RA. *In vitro*, p38 inhibitors have not consistently demonstrated more than modest inhibitory effects on TNF$\alpha$ and IL-1$\beta$-induced cytokine and MMP production by RA FLS (64,69). Many of these inhibitors selectively target p38$\alpha$ and p38$\beta$, and p38$\gamma$ and p38$\delta$ have recently been identified as the predominant p38 isoforms phosphorylated in RA synovial tissue and FLS (70). Involvement of p38$\gamma$ activation in RA may in part explain the lack of therapeutic efficacy of p38 inhibitors in initial clinical trials (71,72). In contrast, p38$\alpha$ appears to be a critical isoform mediating inflammation and joint destruction in murine arthritis (73).

**ERK MAP Kinases**
Of the MAP kinases, a prominent Ras-dependent regulatory role has been most clearly established for ERK 1 and 2 (41,53). ERKs are activated by MEK1 and MEK2, which are substrates for the MEKKs c-Raf/Raf-1, A-Raf and B-Raf. The enzymatic activity of Raf proteins is stimulated by recruitment of the kinases to activated Ras proteins at cellular membranes (53). Like p38, ERK proteins are detected in their activated form in RA synovial tissue (57,58). Pharmacological inhibition of MEK1 and MEK2, or genetic deletion of the KSR scaffolding protein, used by Ras to assemble Raf signaling complexes, is protective in animal models of RA (74,75). However, the pathophysiological contributions of ERK proteins to RA, and animal models of the disease, are poorly characterized. MEK1 and MEK2 inhibitors only weakly diminish TNF$\alpha$-induced cytokine and MMP production by RA FLS (69), although more potent effects have been observed in regard to TRAIL-induced proliferation (76). Dominant-negative
Raf proteins inhibit cytokine-induced RA FLS IL-6 and MMP production, but this strategy likely inhibits not only the Raf/MEK/ERK pathway, but all downstream Ras signaling pathways (39).

**JNK MAP Kinases**

JNK1 and JNK2 are activated in RA synovial tissue, primarily in infiltrating mononuclear cells, but can also be activated in RA FLS following TNFα or IL-1 β stimulation (57,58). MEKK2, MEKK1 and TAK1 can initiate IL-1α -dependent JNK activation in RA FLS (77). This results in activation of MEK-4 and MEK-7 both of which participate in JNK activation. Interestingly, MEK-7 is largely responsible for JNK activation following TNFα or lipopolysaccharide stimulation, while both MEK-4 and MEK-7 are needed for JNK responsiveness to poly (I:C) stimulation of toll-like receptor 3 (78-80). In vitro, pharmacological inhibition of JNK proteins decrease IL-1β-induced collagenase production (58,81). Pharmacological inhibition of JNK proteins is also protective against adjuvant-induced arthritis in rats, while JNK2-deficient mice are resistant to passive antibody-induced arthritis (81,82). The finding that JNK1-deficient mice are resistant to spontaneous arthritis induced in the human TNFα transgenic model of RA has been put forth as an argument that JNK may not be critical to pathology in arthritis, but may also reflect redundant functions of JNK1 and JNK2 in vivo (68).

An important caveat to interpreting all of this experimental data obtained from in vitro studies and animal disease models, is that MAP kinase activation status has only been assessed in RA patients with destructive end-stage arthritis, a patient subgroup which is not participating in clinical trials. RA synovial tissue obtained during arthroscopy and tissue obtained during surgery display differences in both cellular composition and cytokine profiles (83). The activation status of MAP kinases, and their association with clinical parameters of disease activity still needs to be established in patients with active RA.

**Phosphatidylinositol 3- (P13) Kinases**

PI3 kinases consist of a family of lipid kinases which catalyse the phosphorylation of phosphatidylinositol (PI), a minor phospholipids component on the cytosolic side of cell surface membranes. Phosphorylation of PI at the D- position of the inositol headgroup generates the second messengers PtdIns3P, PtdIns(3,4)P2, PtdIns(3,5)P2 and PtdIns(3,4,5)P3 which in turn serve as binding sites which recruit and activate PH domain-containing proteins critical to the promotion of cell growth, cell cycle progression, migration and cell survival (84). The PI3 kinase family is comprised of fourteen enzymes, separated into four classes, of which Class I is the best studied (84). Class I PI3 kinases are further categorized into two subclasses, Class IA and IB. Class IA PI3 kinases are heterodimers consisting of a catalytic subunit (p110 α, β and δ) and a smaller regulatory subunit (p85α, p85β, p55γ, p55α and p50α). There is currently little evidence that specific regulatory subunits preferentially pair with specific catalytic subunits. The regulatory subunit is constitutively bound to p110, and contains two SH2 do-
mains. Class IA PI3 kinases are generally activated by recruitment of the associated adaptor protein to tyrosine-phosphorylated kinase receptors and signalling complexes. Binding of the regulatory subunit SH2 domains to a tyrosine-phosphorylated ligand releases an inhibitory influence of the regulatory protein on p110 (85). Stabilization of p110 activation, and recruitment of the catalytic subunit to its substrate source, is regulated by p110 association with activated Ras proteins (86-89). The requirement for p110 association with Ras proteins is elegantly demonstrated in p110γ knock-in mice expressing only a mutant allele of p110γ which is unable to bind to Ras (90). In contrast to class IA PI3 kinases, the single class IB PI3 kinase, p110γ (associated with p84 or p101 regulatory subunits) is stimulated by heterotrimeric GPCRs (84). All four PI3 kinase p110 catalytic subunits are ubiquitously expressed in mammalian tissue, although p110γ and p110δ expression is enriched in cells of hematopoietic origin.

Accumulation of PI3 kinase products on the cell membrane leads to activation of proteins containing PH domains, amongst which protein kinase B (PKB, also known as Akt) is responsible for many of the PI3-kinase influences on cellular activation, proliferation, and survival (91). One pro-inflammatory function of PKB is the phosphorylation and activation of components of the NFκB pathway (92-94). Additionally, PKB phosphorylates and inactivates three members of the forkhead box O (FoxO) family of transcription factors, FoxO1, FoxO3a, and FoxO4. Depending on other input signals, FoxO family members can promote the transcription of gene products promoting apoptosis, cell cycle arrest, or survival against cellular stress (95). Additionally, recent studies have indicated that FoxO transcription factors can regulate pro-inflammatory genes, such as chemokine receptors, adhesion molecules (96,97) and MMPs (98). FoxO protein transcriptional output appears to be complex, and can have paradoxical effects on cellular responses during inflammation. On one genetic background, FoxO3a-deficient mice demonstrate systemic autoimmune disease, resulting from a T cell lymphoproliferation (99). On another background, mice are protected against antibody-induced arthritis, due to an inability of neutrophils to down-regulate pro-apoptotic Fas ligand (100). Many of the gene targets regulated by FoxO proteins, including manganese superoxide dismutase (MnSOD), growth-arrest and DNA-damage-inducible protein 45 (GADD45), p27, anti-apoptotic Bcl-XL, and pro-apoptotic PUMA are aberrantly expressed in RA synovial tissue, indicating a potential role for FoxO proteins in promoting inflammation in this disease (101).

The first evidence suggesting a role for PI3 kinase signaling in RA came from studies demonstrating the mRNA expression of PTEN, a phosphatase which hydrolyzes PI3 kinase products, was depressed in the intimal lining layer of RA synovial tissue, compared to healthy individuals (21). Decreased PTEN expression in RA synovial tissue is paralleled by a reciprocal increase in PKB activation (102). Pharmacological treatment of RA FLS with relatively non-specific PI3 kinase inhibitors in vitro has suggested a role for PI3 kinase in protecting the cells against TNFα and TRAIL-mediated apoptosis (76,102,103). Furthermore, PI3 kinase signaling promotes RA synovial macrophage survival by promoting expression
of the anti-apoptotic protein Mcl-1 (104), as well as regulating macrophage inflammatory cytokine production (105,106).

A limited number of studies have recently investigated the therapeutic potential of targeting PI3 kinase pathways in vivo. Adenovirus-mediated PTEN gene transfer to rats subjected to CIA decreased angiogenesis, reduced IL-1β and MMP-9 production, and enhanced apoptosis in the synovial tissue of arthritic rats (107). Additionally, PI3 kinase p110γ-deficient mice, and mice treated with specific p110γ inhibitors, are protected against CIA (108). A similar requisite role for p110γ has been observed in the human TNFα transgenic model of RA (109). Potential contributions of other PI3-kinase signaling components to RA remain to be examined.

RAS Signaling and Angiogenesis in RA
Angiogenesis, through neovascularization and vessel remodeling, is critical to the initiation and perpetuation of inflammation in RA (110). Studies in animal models of RA, and changes in endothelial activation observed following successful treatment of RA patients, suggests that direct targeting of angiogenesis may have therapeutic benefit in RA (111-113). Major secreted mediators of angiogenesis in synovial tissue are vascular endothelia growth factor (VEGF) and angiopoietins 1 and 2 (Ang-1, Ang-2). Ang-1, Ang-2, and their shared receptor, the tyrosine kinase Tie2, are all expressed in the synovial tissue of patients with RA and other forms of inflammatory arthritis. (112,114,115). Tie2 has been primarily studied within the context of its expression on endothelial cells and contributions to vascularization during development (116). However, multiple cell populations express Tie2 in inflamed synovial tissue, including endothelial cells, macrophages and FLS (112). Ang-2 stimulation can sensitize endothelial cells to activation by TNFα, while Ang-1 can promote FLS MMP-3 production (117,118). Tie2 is phosphorylated on discreet intracellular tyrosine residues following Ang-1/Ang-2 binding, leading to activation of multiple downstream signaling pathways, including Ras and downstream MAP kinase, PKB, and NFκB pathways (118-121). However, one peculiarity of Tie2 signaling is that Ang-1 and Ang-2 differentially activate each of these downstream signaling pathways, potentially providing a molecular mechanism explaining the distinct contributions of these cytokines to angiogenesis (116).

Outline of This Thesis
In this thesis, we examine the contributions of distinct Ras signaling pathway components to inflammation and joint destruction in RA, using analyses of RA patient synovial tissue, and in vitro and in vivo manipulation of protein expression and activation. Chapter 2 examines the expression of the Ras GEF RasGRF1 in RA synovial tissue and FLS, and the role of this protein in synovial and FLS cytokine and MMP production. In Chapter 3, we explore the expression patterns of distinct Ras family homologues in RA synovial tissue and FLS, and the contributions of these proteins to RA FLS activation, and pathology in the CIA model of RA. Chapter 4 and Chapter 5 provide detailed studies of MAP kinase
expression and activation, and their relationship with disease parameters, in cohorts of patients with longstanding and early arthritis, respectively. In **Chapter 6**, we assess the expression and PKB-dependent phosphorylation of FoxO family transcription factors in synovial tissue and isolated cells relevant to pathology in RA. **Chapter 7** extends studies reported in Chapter 4 to examine the relationship between angiopoietin expression and Tie2 activation in RA and psoriatic arthritis (PsA).
References


chapter 1  Introduction


118. Hashiramoto A, Sakai C, Yoshida K, Tsumiyama K, Mi


