Tyrosine kinases as targets for the treatment of rheumatoid arthritis

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Abstract | As critical regulators of numerous cell signaling pathways, tyrosine kinases are implicated in the pathogenesis of several diseases, including rheumatoid arthritis (RA). In the absence of disease, synoviocytes produce factors that provide nutrition and lubrication for the surrounding cartilage tissue; few cellular infiltrates are seen in the synovium. In RA, however, macrophages, neutrophils, T cells and B cells infiltrate the synovium and produce cytokines, chemokines and degradative enzymes that promote inflammation and joint destruction. In addition, the synovial lining expands owing to the proliferation of synoviocytes and infiltration of inflammatory cells to form a pannus, which invades the surrounding bone and cartilage. Many of these cell responses are regulated by tyrosine kinases that operate in specific signaling pathways, and inhibition of a number of these kinases might be expected to provide benefit in RA.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune synovitis that affects 0.5% of the population and can result in disability owing to joint destruction.[1] A number of cellular responses are thought to be involved in the pathogenesis of RA. An adaptive autoimmune response mediated by T cells and B cells is important in initiating the inflammatory cascade. Macrophages, neutrophils, T cells and B cells migrate into synovial tissue, where they produce immune mediators and degradative molecules that break down the extracellular matrix, in particular that of cartilage. Synoviocytes undergo hyperplasia, and angiogenesis occurs, possibly to support the growth of the synovial lining. Finally, osteoclasts become activated and erode bone. The activation and function of the cell types involved in each of these processes depend on signaling through specific pathways, many of which involve protein tyrosine kinases (Table 1, Figure 1). In support of an important role for tyrosine kinases in RA, proteins from the synovial tissue of RA patients have been found to be extensively phosphorylated by intracellular tyrosine kinases.[2] In this article, we discuss the experimental evidence that implicates specific tyrosine kinases in signaling pathways that are central to the pathogenesis of RA, and address the potential to therapeutically target these kinases. Owing to space limitations, we will not discuss other potential tyrosine kinase targets, including focal adhesion kinase, fibroblast growth factor receptor, epidermal growth factor receptor, and discoidin receptor 2.

Tyrosine kinases

Cell surface and cytoplasmic tyrosine kinases

Tyrosine kinases control many fundamental cell processes, and comprise two general classes of molecules: receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (non-RTKs).[3] In addition to an extracellular ligand-binding domain and a membrane-spanning domain, RTKs usually possess an intracellular cytoplasmic domain that contains a kinase core and regulatory sequences. In the absence of ligands (growth factors, cytokines, etc.), RTKs are thought to exist in an equilibrium of monomers and dimers on the cell surface; ligand binding, however, increases the stability of dimers. Typically, on dimerization, motifs within the intracellular portion of the receptor undergo autophosphorylation, which induces a conformational change that allows the receptor to bind ATP and substrate.[3] The active kinase can then catalyze the transfer of the γ phosphate from ATP to the hydroxyl groups of tyrosine residues on the receptor itself or on substrate proteins. Tyrosine phosphorylation creates docking sites on the receptor for downstream signaling molecules or activates substrate proteins, both of which promote signal transduction (Figure 2).[3]

Similarly, activation of non-RTKs, which lack ligand-binding and transmembrane domains, occurs following phosphorylation of tyrosine residues on proteins by kinases within cytoplasmic complexes. As we will be discussing two members of the Src kinase family—Src and Lck—we will use Src-family kinases to outline non-RTK activation. Members of the Src family have six common structural regions, which, from the N-terminal end, include a Src-homology (SH)-4 domain, a unique region, an SH3 domain, an SH2 domain, a catalytic domain (which comprises an N-lobe and a C-lobe), and a C-terminal tail. Lipid moieties can attach to the SH4 domain to promote plasma membrane localization; the unique region of each family member mediates specific interactions with the cytoplasmic regions of RTKs and other non-RTKs; and the SH3 domain mediates interactions with target signaling molecules. The remaining domains, together with the SH3

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Key points

- Rheumatoid arthritis (RA) is characterized by leukocyte infiltration, synoviocyte hyperplasia and osteoclastogenesis, and tyrosine kinases have key roles in the signaling pathways that regulate these processes
- Inhibition of platelet-derived growth factor receptors, vascular endothelial growth factor receptors and TIE receptors might reduce synovial hyperplasia and angiogenesis
- Inhibition of colony-stimulating factor receptor-1 and Src might reduce monocyte maturation and osteoclastogenesis
- Blocking signaling through Bruton's tyrosine kinase might reduce B-cell and T-cell activation
- Blocking KIT activation might induce mast cell apoptosis, thereby reducing the production of inflammatory cytokines and degradative molecules in the synovium
- Imatinib, which inhibits several tyrosine kinases, and more-specific inhibitors of Janus kinases and Syk, have already shown efficacy in the treatment of RA; however, toxicity remains an issue

domain, modulate the activation of Src-family kinases using two critical tyrosine residues—tyrosine 416 in the catalytic domain and tyrosine 527 in the C-terminal tail (the numbering refers to Src)—with opposing effects. Normally, Src-family kinases reside in an inactive conformation, in which the molecule is folded upon itself such that the activation loop (which contains tyrosine 416) is buried between the two lobes of the catalytic domain. This folded configuration is maintained by interactions between the SH3 domain and the linker region between the SH2 and catalytic domains, and by the binding of the SH2 domain to the C-terminal tail following phosphorylation of tyrosine 527. Activation occurs when interactions between the SH2 and SH3 regions and high-affinity binding ligands disrupt the intramolecular interactions, allowing the molecule to unfold to expose tyrosine 416 on the activation loop for autophosphorylation. Phosphorylation of this residue activates the kinase.[4]

Inhibitors of tyrosine kinases

A large number of small-molecule tyrosine kinase inhibitors (TKIs) have been developed. These inhibitors typically, but not always, bind to the nucleotide-binding pocket of the catalytic domain, and can thereby modulate changes in the conformation of the molecule that are necessary for kinase activation. For instance, imatinib is a potent inhibitor of several tyrosine kinases, including KIT (also known as CD117), platelet-derived growth factor receptors α and β (PDGFRα and β), breakpoint cluster region (Bcr)–Abelson murine leukemia viral oncogene homolog 1 (ABL1), colony-stimulating factor 1 receptor (CSF1R), and leukocyte-specific protein tyrosine kinase (Lck). Imatinib blocks the function of Bcr–ABL, and possibly these other tyrosine kinases, by stabilizing their inactive conformation.[5]

FLS hyperplasia

RA is characterized by hyperplasia of the synovial lining.[1] Synovial tissues derived from RA patients exhibit a marked increase in the number of macrophage-like and fibroblast-like synoviocytes (FLSs).[1,6]

PDGFRs in FLS proliferation

Key molecules that are involved in FLS proliferation are PDGFRs, of which there are two (PDGFRα and β),[7] and their ligands, which are dimers formed from PDGFs A–D.[2,8] Increased levels of PDGFRα transcripts and proteins are seen in FLSs cultured from RA patients compared with those taken from control patients; PDGFRβ is expressed on stromal cells in the synovial lining, and in smooth muscle cells and capillary cells in RA synovium (Table 1).[8–10]

PDGFRs as potential targets

In vitro, PDGF induces proliferation of synovial fibroblasts derived from patients with RA more potently than synovial fibroblasts derived from healthy individuals.[9] In rodent models of RA, imatinib reduced the severity of symptoms when administered before the development of the disease and inhibited disease progression when given to mice that had already developed the disease.[11–13] In addition, nilotinib, which inhibits Bcr–ABL, PDGFRα and β and KIT, was effective in treating arthritis in the K/BxN serum-induced model (C. D'Aura Swanson *et al.*, unpublished data). The efficacy of imatinib (and nilotinib) in models of arthritis is probably attributable, at least in part, to inhibition of PDGFR, as several groups have shown that imatinib inhibits the proliferation of FLSs from RA patients by blocking the phosphorylation of PDGFR and the activation of downstream mediators of the PDGFR signaling pathway.[14,15] Evidence to date, therefore, indicates that PDGFRα and β have a central role in the proliferation of FLSs, and thereby represent potential therapeutic targets in RA.

Angiogenesis

Vascular density is higher in the synovia of patients with RA and osteoarthritis (OA) than in healthy synovium.[16] Increased vascularity might support the growth of the synovial lining, so inhibiting angiogenesis is an attractive therapeutic approach for the treatment of arthritis. Such inhibition might be achieved by targeting vascular endothelial growth factor receptors 1 and 2 (VEGFR1 and 2) and TIE1 and TIE2 (also known as TEK).

VEGFs and VEGFRs as potential targets

VEGFRs comprise the most-studied family of RTKs in RA. The family includes VEGFR1 (also known as Flt-1 or Flk-2), VEGFR2 (known as KDR in humans and Flk-1 in mice), VEGFR3 (Flt-4), and two receptors that lack kinase domains: neuropilin (NRP)1 and NRP2.[17] Ligands for the VEGFRs include VEGFs A–F and placental growth factor.[17] Several studies have demonstrated an increase in the expression of VEGFA protein in the synovial fluid, lymph, serum and synovial tissue of patients with RA. The levels of serum VEGFA positively correlate with RA disease activity.[18–20] VEGFA activates VEGFR1 and VEGFR2, which, in turn, induces endothelial cell proliferation, sprouting, migration and tube formation, thereby promoting the generation of blood vessels.[17] VEGFA also supports osteoclastogenesis by mimicking the actions

Table 1 | Expression of select tyrosine kinases in human rheumatoid arthritis patients

Name	Type	Ligands	Expression in human RA synovium	Roles in RA
PDGFRα[8]	RTK	PDGFAA,BB,CC,DD,AB	Synovial fluid	FLS proliferation
PDGFRβ[10]	RTK	PDGFAA,BB,CC,DD,AB	Lining, blood vessels	FLS proliferation, angiogenesis
VEGFR1[17,24,25]	RTK	VEGFA–D	Monocytes/macrophages of sublining, blood vessels, pannus	Angiogenesis
VEGFR2 (Flk-1 in mice)[23, 24]	RTK	VEGFA–D	Blood vessels, pannus	Angiogenesis
VEGFR3[23]	RTK	VEGFA–D	Blood vessels	Lymphangiogenesis
TIE1[32]	RTK	ANG1–4	Lining, monocytes/macrophages of sublining, and blood vessels	Angiogenesis
TIE2[32]	RTK	ANG1–4	Lining, monocytes/macrophages and lymphocytes of sublining and blood vessels	Angiogenesis
KIT[43]	RTK	SCF	Mast cells of sublining	Production of inflammatory cytokines and MMPs
CSF1R[51]	RTK	M-CSF	Lining, monocytes/macrophages of sublining and blood vessels	Macrophage maturation, osteoclastogenesis
Lck[38]	Non-RTK	NA	Lymphocytes of sublining	Production of inflammatory cytokines
Btk[39,a]	Non-RTK	NA	Lymphocytes and mast cells of sublining	Activation of B cells, monocytes/ macrophages and mast cells
Syk[76]	Non-RTK	NA	Lining and synovial fluid	B-cell activation
Src[64, 65]	Non-RTK	NA	Lining, monocytes/macrophages and mast cells of sublining and synovial fluid	Migration of monocytes/ macrophages and FLSs, osteoclastogenesis
JAK3[73]	Non-RTK	NA	Lining, monocytes/macrophages and lymphocytes	T-cell activation

[a]Molecule expression examined in normal tissue, but not examined in RA tissue. Abbreviations: ANG, angiopoietin; CSF1R, colony-stimulating factor 1 receptor; FLS, fibroblast-like synoviocyte; JAK, Janus kinase; M-CSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; NA, not applicable; PDGF, platelet-derived growth factor; RA, rheumatoid arthritis; RTK, receptor tyrosine kinase; SCF, stromal cell factor; VEGF, vascular endothelial growth factor.

of macrophage colony-stimulating factor (M-CSF; also known as CSF1).[17,21] Certain VEGFA isoforms and NRP1 induce the upregulation in synoviocytes of B-cell leukemia/ lymphoma 2 (Bcl-2), which can protect the cells against apoptosis and thereby promote hyperplasia.[22] VEGFC and VEGFD increase vascular permeability and angiogenesis by signaling through VEGFR2.[17] VEGFC is present in many cells in the thickened synovial lining in RA, especially in blood vessel pericytes and smooth muscle cells.[23] Endothelial cells, monocytes/macrophages and osteoblasts in the synovial tissue of RA patients express VEGFR1 (Table 1), but the levels of this protein do not correlate with vascularity.[17,24,25] VEGFR2 can be detected in endothelial cells, especially in small blood vessels in pannus tissue, and its expression is upregulated in the synovial tissue of RA patients compared to that of OA patients (Table 1).[22,23,25]

Several groups have shown that inhibition of VEGFA or VEGFR1 delays the onset and reduces the severity of murine arthritis.[26–29] VEGFR2 is thought to mediate VEGFA-induced endothelial cell migration and proliferation, but the value of inhibiting VEGFR2 or VEGFR3 in arthritis models remains unclear.[17] Inhibition of VEGFR2 has yielded mixed results, whereas pan-VEGF inhibitors have shown only moderate efficacy.[26,28,29] Sorafenib, which inhibits multiple tyrosine kinases, including VEGFR2 and VEGFR3, showed moderate therapeutic efficacy against murine K/BxN serum-induced arthritis, but failed to reduce disease scores or paw swelling in murine collagen-induced arthritis (CIA) (C. D'Aura Swanson *et al.*, unpublished data). Sunitinib, which inhibits VEGFR1, VEGFR2 and VEGFR3, as well as several other kinases,[30] reduced the incidence and severity of both K/BxN serum-induced arthritis and CIA (C. D'Aura Swanson *et al.*, unpublished data). However, because sorafenib and sunitinib inhibit more than one form of VEGFR, more-specific inhibitors or genetic manipulation of the individual VEGFRs is required to determine the importance of the different VEGFRs in arthritis models; thus, further research is needed to clarify the roles of individual VEGFRs in RA. Nevertheless, findings to date indicate that VEGFR1 and VEGFA are prime candidates for therapeutic intervention in RA.

TIE1 and TIE2 as potential targets

The RTKs TIE1 and TIE2 regulate angiogenesis and are expressed on endothelial cells.[31] Their ligands are the angiopoietins 1–4:[31] angiopoietin 1 is a potent TIE2 agonist, angiopoietin 2 can function as a TIE2 agonist or antagonist, depending on cell context, and TIE1 regulates TIE2 activity.[31] TIE1, TIE2, angiopoietin 1 and

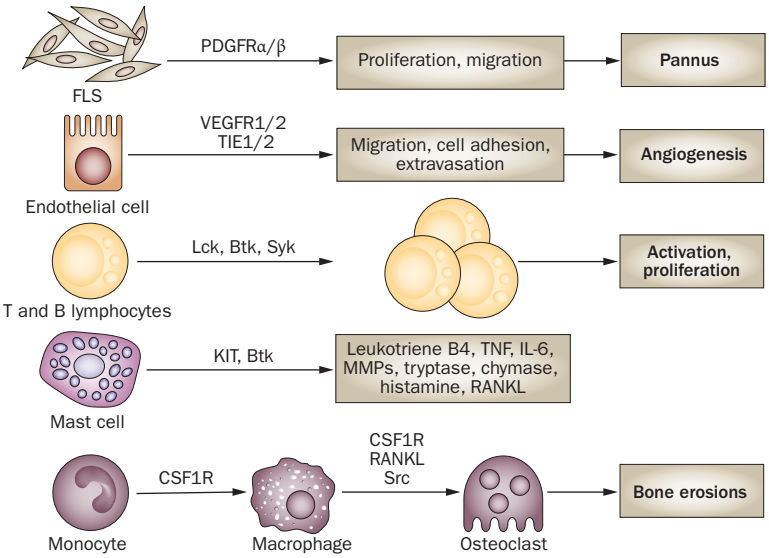

Figure 1 | Cellular responses mediated by tyrosine kinases that contribute to the pathogenesis of rheumatoid arthritis. Signaling through PDGFRs promotes the proliferation and migration of FLSs, contributing to the formation of a pannus. Migration of endothelial cells to form blood vessels through angiogenesis is promoted by signaling through VEGFRs and regulated by TIE1 and TIE2. Activation of T cells and B cells through T-cell receptors and B-cell receptors, respectively, requires a variety of tyrosine kinases, including Lck, Btk and Syk. Mast cells, which produce numerous inflammatory and degradative factors in the synovium, can be activated by several routes, such as binding of SCF to KIT. M-CSF binding CSF1R promotes the maturation of monocytes into macrophages and subsequent osteoclast formation, which results in bone erosion. Abbreviations: CSF1R, colony-stimulating factor 1 receptor; IL-6, interleukin-6; FLS, fibroblast-like synoviocyte; M-CSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; PDGFR, platelet-derived growth factor receptor; RANKL, receptor activator for nuclear factor κB ligand; SCF, stem cell factor; TNF, tumor necrosis factor.

angiopoietin 2 are all expressed in synovial tissue from RA patients,[32,33] and inhibition of TIE1 or TIE2 signaling was shown to be beneficial in CIA (Table 1). Adenovirus-mediated overexpression of a soluble form of TIE2, which functions as an antagonist of TIE2 signaling, significantly decreased disease incidence and severity in a CIA model; these results were associated with a reduction in angiogenesis, synovial cell infiltration and radiographic paw scores.[34] TIE1-751, a naturally occurring splice variant of TIE1, also reduced arthritis severity in CIA.[28] TIE1 and TIE2, therefore, represent potential targets in RA therapy.

T-cell and B-cell activation

The importance of T cells and B cells in the pathogenesis of RA is underscored by several findings: the genetic linkage of RA to HLA genes; the association of RA with autoantibodies; the success of a fusion protein that inhibits T-cell costimulation (CTLA4–Ig; abatacept) in an RA clinical trial; and the efficacy of B-cell depletion by rituximab in the treatment of RA patients.[35,36]

Kinase involvement

Activation of T cells and B cells through T-cell receptors (TCRs) and B-cell receptors (BCRs), respectively, requires a variety of tyrosine kinases, including Lck, spleen tyrosine kinase (Syk), and Bruton's tyrosine kinase (Btk).[37] Inhibition of Lck disrupts TCR signaling, and thereby reduces T-cell activation, proliferation, and cytokine production.[38] Btk is primarily expressed in B cells, mast cells, platelets and myeloid cells.[39] This tyrosine kinase mediates calcium signaling following BCR engagement, mast cell activation following FcεRI crosslinking, and possibly monocyte activation following engagement of Toll-like receptor 4.[39,40]

Lck, Syk and Btk as potential targets

Two Btk inhibitors, Compound 4 (Celera Genomics, Alameda, CA) and cgi1746 (CGI Pharmaceuticals, Branford, CT), have shown efficacy in collagen antibody-induced arthritis (CAIA) and in CIA, respectively (Table 2).[39,41] Preventive treatment with dasatinib, which potently inhibits Lck and Btk in addition to other tyrosine kinases,[42] reduced the incidence and severity of both CIA and K/BxN serum-induced arthritis (C. D'Aura Swanson *et al.*, unpublished data). Syk will be discussed in further detail below.

Mast cells

Activation and function

Mast cells produce factors that regulate a number of functions, including lymphocyte migration, angiogenesis, inflammation, and cartilage and bone destruction.[43,44] Mast cells enhance vascular permeability through the release of bradykinin, and produce leukotriene B4, which might recruit $CD8^+$ effector cells.[43,44] Through the production of heparin, basic fibroblast growth factor, tumor necrosis factor (TNF), interleukin (IL)-13, IL-1β, IL-8, VEGF and PDGF, mast cells promote angiogenesis; by producing tryptase, chymase, histamine and receptor activator for nuclear factor κB ligand (RANKL; also known as TNF ligand superfamily, member 11), these cells also promote the destruction of extracellular matrix and bone.[43,44] Mast cells constitute up to 3% of cells in healthy synovium, but this figure can increase to 5% in murine and human arthritic synovial tissue.[43,44] In the synovial tissue of RA patients, mast cells are observed at the cartilage–pannus junction, near blood vessels, and in areas of fibrosis.[44] Molecules that inhibit mast cell degranulation decrease joint swelling in murine models of arthritis;[44] W/Wv mice, which are deficient in mast cells, are resistant to K/BxN serum-induced arthritis.[45]

Targeting KIT

Signaling through FcεRI, FcγRIII, KIT and complement receptors probably activates mast cells in RA.[43,44] Because mast cell activation can occur through several distinct signaling pathways, blocking this activation can be challenging. One strategy to reduce the production of mast cell effector molecules is to induce apoptosis in these cells; indeed, imatinib has been shown to reduce the number of cultured mast cells via apoptosis.[46] The KIT ligand (also known as stem-cell factor [SCF]) is thought to be one of the most important growth factors for mast cells.[43,44] SCF

is found in increased quantities in the synovial fluid of RA patients compared to that of OA patients,[47] is expressed on multiple cells in the synovium,[48] and induces a strong chemotactic response in mast cells from RA patients.[49] KIT represents, therefore, a logical target for RA therapy, especially given its restricted expression in mast cells.

Osteoclast-mediated destruction

Osteoclasts erode periarticular bone in RA.[21] Their formation from monocytes and dendritic cells is regulated by RANKL and M-CSF. Monocytes from synovial fluid from the joints of RA patients form large, multinucleated, tartrate-resistant and acid phosphatase-positive cells (which is indicative of the presence of osteoclasts) on culture with M-CSF and RANKL.[50] M-CSF, through its ability to induce Bcl-2 and activate Ras-related C3 botulinum toxin substrate 1, also promotes osteoclast survival and the formation of resorptive pits.[21]

CSF1R as a potential target

M-CSF and its receptor CSF1R (a product of the *fms* gene) are expressed in the synovium of RA patients. CSF1R protein expression is observed in the synovial lining layer (predominantly on $CD68^+$ macrophages) and around vessels in the sublining, as well as in osteoclasts (Table 1).[51] Endothelial cells and fibroblasts from RA patients express high levels of M-CSF,[52] and M-CSF levels are increased in synovial tissues and fluid derived from RA patients.[53]

Findings from rodent models of arthritis indicate that M-CSF and CSF1R have crucial roles in disease pathogenesis and progression. M-CSF exacerbates arthritis induced by submaximal levels of collagen or methylated bovine serum albumin in mice,[54,55] and in rat arthritis.[56] Mice deficient in M-CSF (*op/op* mice) are resistant to the development of arthritis,[57] whereas antibodies against M-CSF reduced the clinical severity of CIA,[54] and neutralizing antibodies targeting CSF1R inhibited inflammatory osteolysis.[58] Furthermore, the small-molecule CSF1R inhibitors GW2580 (GlaxoSmithKline, Uxbridge, UK) and Ki20227 (Kirin Pharma Company Ltd, Takasaki, Japan) show therapeutic efficacy in rodent models of arthritis.[59,60] Several pharmaceutical companies are developing CSF1R-specific inhibitors.

Src as a potential target

Src is a ubiquitously expressed non-RTK that is activated, among other means, by binding to protein tyrosine kinase 2β (PTK2β; also known as Pyk2) following integrin activation.[61] Integrins, such as $\alpha_v\beta_3$, are critical for bone resorption, as they are thought to mediate macrophage and osteoclast migration and osteoclast adhesion to bone,[62] and they promote osteoclast survival.[63] Src is also important in RANK signaling, as interaction of Src with TNF receptor-associated factor 6 (TRAF6) following RANK receptor engagement leads to the phosphorylation of downstream signaling molecules.[64] In RA patients, cells of the synovial lining and subsynovial macrophages express phosphorylated (activated) Src.[65]

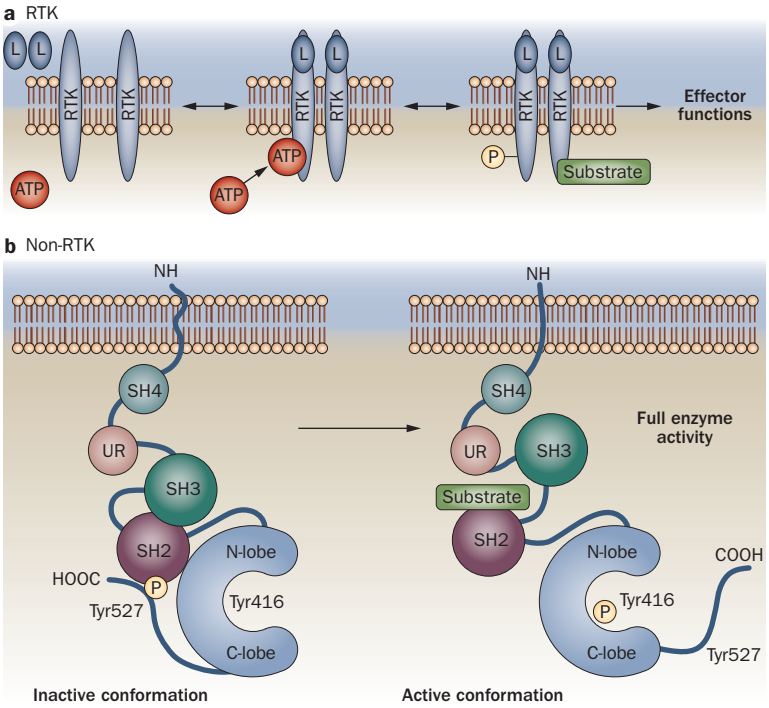

Figure 2 | Activation of tyrosine kinases. There are two main types of tyrosine kinase: RTKs and non-RTKs. **a** | Ligand binding increases the stability of transmembrane RTK dimers; motifs within the intracellular domain undergo autophosphorylation, inducing a conformational change that allows ATP and substrate binding.[3] The active kinase catalyzes the transfer of γ-phosphate from ATP to hydroxyl groups of tyrosine residues on the receptor itself or on substrate proteins, creating binding sites or activating substrate proteins, respectively, to promote signal transduction. **b** | Src-family kinases normally adopt an inactive conformation: the molecule is folded upon itself, such that the activation loop, containing tyrosine 416, is buried between the N-lobe and C-lobe of the kinase domain. This configuration is maintained by interactions between the SH3 domain and the linker region connecting the SH2 with the catalytic domain, and by binding of the SH2 domain to the C-terminal tail following phosphorylation of tyrosine 527. Activation occurs when interactions between the SH2 and SH3 regions and high-affinity binding ligands disrupt these intramolecular interactions, allowing unfolding and revealing tyrosine 416 for autophosphorylation. The mechanism of Src activation is outlined here. Abbreviations: L, ligand; RTK, receptor tyrosine kinase; SH2, SH3 and SH4, Src-homology 2, 3 and 4 domains; UR, unique region.

Targeted disruption of Src in mice induces osteopetrosis, which is characterized by decreased bone resorption.[62] In addition, overexpression *in vivo* of C-terminal Src kinase (Csk), which negatively regulates Src and other Src-family kinases, reduced the expression of these kinases and decreased arthritis severity in rats.[66]

Chemokine and cytokine production

TNF and IL-1 have key roles in RA as evidenced by the efficacy of inhibitors of both of these molecules in the treatment of RA.[67,68] Moreover, TNF and IL-1 signaling pathways can synergize with, or activate, tyrosine kinases. Specifically, PDGF can synergize with IL-1 and TNF to promote fibroblast proliferation and prostaglandin E2 production.[69] Similarly, IL-1 and TNF stimulate the production of M-CSF by cartilage and fibroblasts.[70] Blockade of TNF and IL-1 might, therefore, provide benefit in RA

Table 2 | Small-molecule inhibitors of select tyrosine kinases

Generic name	Described kinase targets	Stage of clinical development	Primary disease indication
Imatinib[11,14,46]	KIT, PDGFRα, PDGFRβ, Bcr–ABL, CSF1R, Lck	Approved	CML, GIST
Dasatinib[38,42]	FAK, Fyn, Yes, Lck, Src, Bcr–ABL, Lyn, EphB4, Btk, DDR1, KIT, PDGFRβ, DDR2, Tec	Approved	CML
Sorafenib[30]	PGFRβ, VEGFR2, VEGFR3, KIT, CSF1R, Flt-3, Raf1, B-Raf	Approved	Renal cell carcinoma and hepatocellular cancer
Sunitinib[30]	VEGFR1, VEGFR2, VEGFR3, PDGFRβ, PDGFRα, KIT, Flt-3, CSF1R	Approved	Renal cell carcinoma
Nilotinib[30]	Bcr–ABL, PDGFRs, KIT, Lck	Approved	CML
CP-690550 (Pfizer Inc.)[73]	JAK3	Phase II	RA
INCB18424 (Incyte Inc.)[74]	JAK1, JAK2	Phase II	RA
Fostamatinib disodium (R406/R788; Rigel Pharmaceuticals Inc.)[76]	Syk	Phase II	RA
Compound 4 (Celera Genomics)[39]	Btk	Preclinical	NA
Cgi1746 (CGI Pharmaceuticals Inc.)[41]	Btk	Preclinical	NA
GW2580 (GlaxoSmithKline plc)[59]	CSF1R, TrkA	Preclinical	NA
Ki20227 (Kirin Pharma Company Ltd.)[60]	CSF1R, VEGFR2, KIT, PDGFRβ	Preclinical	NA

Abbreviations: Bcr–ABL, breakpoint cluster region–Abelson; CML, chronic myelogenous leukemia; CSF1R, colony-stimulating factor-1 receptor; DDR, discoidin domain receptor; FAK, focal adhesion kinase; JAK, Janus kinase; GIST, gastrointestinal stromal tumor; NA, not applicable; PDGF, platelet-derived growth factor; RA, rheumatoid arthritis; VEGFR, vascular endothelial growth factor receptor.

by reducing the production of synovial M-CSF and effects of PDGF.

TKIs and RA

Eight small-molecule TKIs have so far been approved by the FDA for the treatment of different types of cancer; five of these TKIs might provide benefit in the treatment of RA (Table 2). Several TKIs are also in clinical development for the treatment of RA (Table 2). A number of case reports and a small case series indicate that imatinib, which is approved for the treatment of cancer, might provide benefit in RA and other inflammatory diseases. These reports include studies involving patients with long-standing RA who developed Bcr–ABL-positive chronic myelogenous leukemia[71] or KIT-positive gastrointestinal stromal tumor.[72] These patients received imatinib (300–400 mg daily) for their malignancy and showed improvement in RA symptoms, as reflected by reductions in the levels of inflammatory markers and improvements in disease activity indices.[72] On the basis of these results, Eklund and Joensuu[72] administered open-label imatinib treatment to three patients who had treatment-refractory RA. All three patients experienced some degree of clinical improvement, as assessed by measurement of inflammatory markers and swollen and tender joint counts; however, one patient discontinued therapy owing to the development of a rash.[72]

Inhibitors that target other tyrosine kinases with potential involvement in RA, such as members of the Janus kinase (JAK) family[73] (CP-690550 [Pfizer Inc., New York, NY], INCB18424 [Incyte Inc., Wilmington, DE])[74,75] or Syk[76] (fostamatinib disodium [R788; Rigel Pharmaceuticals Inc., San Francisco, CA]),[77] have undergone or are currently undergoing phase II clinical trials for the treatment of this disease (Table 2). The Syk inhibitor fostamatinib disodium showed significant improvements in ACR response and 28-joint disease activity score (DAS28) in a phase II study.[77] Nevertheless, the use of Syk inhibitors might be limited by toxicity-related adverse effects that result in hepatic inflammation and hypertension. It remains unclear whether these toxicities are the result of a class effect or a molecule effect.[77]

Conclusions

Scientists and clinical researchers have begun to investigate small-molecule TKIs as a novel therapeutic approach to RA and other inflammatory diseases. Of the tyrosine kinases discussed in this Review, those that currently show the greatest potential as therapeutic targets in RA are CSF1R and KIT. The expression of these kinases is restricted to specific cell types within the synovium, and cumulative evidence from studies on tissues from RA patients and from animal models, as well as the efficacy of imatinib in treating RA, point to a central role for CSF1R and KIT in RA pathogenesis. However, as our knowledge regarding the roles of other tyrosine kinases in RA increases, their importance as targets might also be fully appreciated.

In vitro and *in vivo* observations involving both rodent models and samples derived from RA patients indicate that targeting the kinases discussed herein might provide benefit in the treatment of RA. However, several issues remain. First, although each of these molecules seems to have an important role in the pathogenesis of RA, their

relative contributions need to be further defined. Second, the therapeutic effect (or lack thereof) of TKIs in animal models cannot reliably be extrapolated to human RA patients.[78] Third, TKIs such as imatinib and dasatinib are pleiotropic inhibitors—they might additionally inhibit tyrosine kinases that are not involved in RA, and thus be more likely to cause unrelated tissue damage. Tyrosine kinases have central roles in many physiologic processes: for example, PDGFR regulates fibroblast proliferation and wound healing,[79] and CSF1R regulates monocyte lineage cell survival and differentiation.[80] Small-molecule TKIs will partly block several of these physiologic responses, and might thereby cause target-based toxicities that limit their therapeutic use, as illustrated by recent clinical trials involving Syk inhibitors.[77] It is difficult to fully anticipate the toxicities and therapeutic benefits that might arise from the inhibition of a particular tyrosine kinase or set of tyrosine kinases.

Although we focus on small-molecule inhibitors in this Review, other methods exist to target tyrosine kinases in RA. For example, antibodies targeting the extracellular domains of RTKs could compete with ligand binding or interfere with the adoption of molecular conformations necessary for activation. In addition, small interfering RNAs might be used to downregulate the expression of specific tyrosine kinases.

Specific targeting of tyrosine kinases that have central roles in the pathogenesis of RA will need to be carried out in clinical trials. The severity of disease and the adverse effects of a given TKI must be carefully considered. Given the breadth of therapeutics that are already available for the treatment of RA, it will be essential to identify TKIs with therapeutic indices that are sufficient for their use in RA.

Review criteria

PubMed was searched in November 2008 using the following PubMed terms, alone and in combination, to identify original articles in the literature, published in English, on tyrosine kinases in rheumatoid arthritis: "rheumatoid arthritis", "tyrosine kinase", "imatinib", "sorafenib", "sunitinib", "dasatinib", "nilotinib", "Syk", "JAK", "PDGF", "VEGF", "TIE", "Src", "KIT", and "M-CSF". We cited data frequently from primary studies, but also included information from reviews, conference abstracts and press releases.

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