Phosphoinositide 3-kinase signalling and FoxO transcription factors in rheumatoid arthritis

K.A. Reedquist¹, J. Ludikhuize and P.P. Tak
Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Room K0-140, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Abstract
Although the mechanisms leading to the induction of RA (rheumatoid arthritis) are poorly understood, improper activation, proliferation, survival and retention of neutrophils, macrophages, lymphocytes and other leucocytes contribute to perpetuation of inflammation and eventual joint destruction through activation of stromal fibroblast-like synoviocytes. Fundamental studies in developmental biology, cellular biology and immunology have established critical roles for PI3K (phosphoinositide 3-kinase) signal transduction pathways in cellular chemotactic responses, proliferation, apoptosis and survival. Despite profound alteration of these cellular processes in RA, involvement of PI3K signalling pathways in this chronic inflammatory disease, and their assessment as potential therapeutic targets, has until recently received scant attention. This review highlights recent advances in our understanding of PI3K signalling pathways, in particular regulation of FoxO (forkhead box O) transcription factors, and their relevance to RA.

RA (rheumatoid arthritis): chronic inflammation and chronic signal transduction
RA is a common, progressive, destructive inflammatory disease characterized by improper activation, proliferation, survival and retention of neutrophils and lymphoid cells recruited to affected joints. Infiltrating immune cells perpetuate inflammation and promote joint destruction via interactions with stromal FLS (fibroblast-like synoviocytes) [1]. FLS cell–cell contact with immune cells, or FLS exposure to growth factors and inflammatory cytokines, such as TNF-α (tumour necrosis factor α) and IL-1 (interleukin-1), triggers FLS production of potent chemokines, angiogenic and pro-inflammatory growth factors, and matrix metalloproteinases and cathepsins that degrade extracellular matrix and cartilage [2].

The mechanism(s) leading to the initiation of RA are poorly understood, but strong evidence indicates that defects in cellular apoptosis contribute to the accumulation and persistence of immune cells, both adaptive and innate, as well as FLS in RA synovial tissue [3]. Histological examination of synovial hyperplasia in RA, and similarities in vitro between transformed cell lines and RA FLS (e.g. anchorage-independent growth, autocrine production and invasive properties) suggested that acquired lesions in key proto-oncogene or tumour suppressor genes may contribute to pathology in RA [2,4,5]. While somatic mutations in genes such as those encoding Ras and PTEN (phosphatase and tensin homologue deleted on chromosome 10) have not been identified, it is now clear that persistent activation of intracellular signalling pathways regulated by cell–cell contacts and the cocktail of growth factors, chemokines and inflammatory cytokines present in RA synovial tissue. Important roles for mitogen-activated protein kinase and NF-κB (nuclear factor κB) signalling pathways in RA are well-documented, and intensive efforts are being made to target these pathways therapeutically [6,7]. In contrast, despite the wealth of fundamental studies showing a central role for PI3K (phosphoinositide 3-kinase) pathways in modulating cellular survival and apoptosis, relatively little is known about the contribution of PI3K signalling to pathology in RA.

PI3K signalling pathways
PI3Ks are intracellular heterodimeric enzymes consisting of regulatory and p110 catalytic subunits. Class IA PI3Ks (α, β and δ) are stimulated by recruitment of regulatory subunits to activated growth factor, cytokine and antigen receptors, while Class IB PI3Kγ is stimulated by direct binding of the catalytic subunit to activated G-protein-coupled receptors, such as chemokine receptors [8,9]. Activation of both Class IA and IB PI3Ks may be facilitated by catalytic subunit association with activated Ras family GTPases. The resulting generation of phosphorylated lipid metabolites recruits and stimulates proteins containing PH (pleckstrin homology) domains. PI3K signalling is attenuated by the tumour suppressor lipid phosphatase PTEN. PH domain-containing targets of
PI3K signalling include activators of Rho family GTPases, involved in chemotactic responses, PDK1 (phosphoinositide-dependent kinase 1), and PKB (protein kinase B; also referred to as Akt).

PKB activation is responsible for many of the effects of PI3K signalling on cellular activation, proliferation and survival, through phosphorylation-dependent regulation of IKK (IκB [inhibitory κB] kinase), the pro-apoptotic Bcl-2 family protein Bad (Bcl-2/Bcl-X<sub>L</sub>-antagonist, causing cell death), glycogen synthase kinase-3, mTOR (mammalian target of rapamycin) signalling and FoxO (forkhead box O) transcription factors [10]. Three FoxO family members, FoxO1, FoxO3a and FoxO4, are inactivated by PKB-dependent phosphorylation on conserved residues. PKB-dependent phosphorylation directly interferes with FoxO binding to target DNA sequences and promotes FoxO association with 14-3-3 proteins mediating FoxO nuclear export and exclusion [11]. In the absence of PKB signalling, active FoxO proteins promote transcription of a number of genes that regulate cell-cycle progression, apoptosis, and survival, examples of which include FasL, Bim, Bcl-6, p27Kips, cyclin D proteins and MnSOD (manganese-dependent superoxide dismutase) [11].

**PI3K signalling in murine inflammatory disease**

The potent inflammatory consequences of deregulated PI3K signalling are readily observed in gene-targeting studies in mice [8,12]. Heterozygous inactivation of PTEN or constitutive activation of PKB in the T-cell compartment leads to a polyclonal expansion of activated B- and T-lymphocytes that infiltrate multiple organs and cause lethal autoimmune disease [13,14]. A direct role for FoxO proteins in autoimmune disease is observed in FoxO3a-deficient mice, which display age-dependent T cell lymphoproliferation, associated with multi-organ inflammation [15].

Reciprocally, inhibition of PI3K signalling pathways blunts lymphocyte immune responses and protects animals in experimental models of arthritis. T- and B-lymphocytes from mice expressing catalytically inactive PI3K p110δ display defects in maturation and antigen receptor-dependent proliferative responses [16]. Genetic deletion of p110δ impairs B-cell chemotactic responses, while p110γ deletion prevents chemotactic responses by leukocytes and T-cells [17,18]. In the passive, anti-collagen antibody-induced model of arthritis, a disease primarily dependent on leucocyte recruitment and activation, p110γ-deficient mice and mice treated with a selective pharmacological inhibitor of p110γ are protected from inflammation and cartilage destruction [18].

The pharmacological inhibitor also blocks the induction of collagen-induced arthritis, and ameliorates established arthritis in this model. The latter finding is particularly exciting in the clinical context, where strategies to treat established RA, long after the onset of inflammation, are needed. As initial attempts to treat RA with chemokine receptor blockades have demonstrated promising efficacy [19], further development of p110 isoform-specific inhibitors for blocking accumulation of activated immune cells in the RA synovium is warranted.

**PI3K regulation of cellular activation, proliferation and survival in RA**

Direct studies of the contribution of PI3K to the pathology of RA are in their infancy. The first indication that PI3K signalling pathways may be involved in RA stems from the observation that PTEN mRNA expression is decreased in cells composing the invasive intimal lining layer of the RA synovium, and in RA FLS actively invading human cartilage in the severe combined immunodeficiency murine arthritis model [20]. Together, these results suggested that PI3K signalling may be elevated in RA. PI3K-dependent phosphorylation of PKB is significantly higher in RA synovial tissue and FLS than in tissue and cells derived from osteoarthritis patients [21]. In vitro, RA FLS PKB is activated by TNFα stimulation, and introduction of dominant-negative PKB, or overexpression of PTEN, facilitates TNFα-induced apoptosis. The protective effects of TGFβ against Fas-induced apoptosis in FLS are associated with PKB activation and are abrogated with the PI3K inhibitor LY294002 [21,22]. LY294002 also blocks TRAIL (TNF-related apoptosis-inducing ligand) activation of PKB, sensitizes FLS to TRAIL-induced apoptosis, and blocks subsequent TRAIL-dependent proliferation of surviving cells [23,24]. PI3K-dependent responses of RA FLS are not limited to survival decisions, as LY294002 can suppress cytokine-dependent up-regulation of IL-6, IL-8 and VCAM-1 (vascular cell adhesion molecule 1) expression [25,26].

PI3K signalling pathways similarly regulate survival and activation of RA synovial macrophages. Constitutive activation of PKB promotes survival in human macrophages via up-regulation of the anti-apoptotic Bcl-2 family protein Mcl-1 [27]. PI3K signalling also influences the balance of pro- and anti-inflammatory cytokines secreted by RA synovial macrophages, where LY294002 treatment simultaneously inhibits spontaneous IL-10 secretion and enhances TNFα production [28–30].

At least some of the effects of PKB in RA may be attributable to its activation of the mTOR/p70 S6-kinase pathway. In vitro, the mTOR inhibitor rapamycin can block platelet-derived growth factor and insulin-like growth factor-1-induced RA FLS proliferation and IL-6 production, as well as inducing apoptosis [31–33]. Additionally, rapamycin can block macrophage activation in response to synovial T-lymphocytes [28]. Despite effective widespread use of mTOR signalling inhibitors as immunosuppressants in transplant medicine, clinical data on the effects of targeting mTOR in arthritis are limited to case reports of RA and juvenile RA patients undergoing organ transplant [34,35].

**Modulation of FoxO transcriptional targets in RA**

A role for PKB-dependent inactivation of FoxO proteins in RA is at the moment only speculative, as no assessment of the
presence, modification, or transcriptional activity of FoxO proteins in RA synovial tissue, or cells derived from patients has been reported. However, independent reports of modulation of known FoxO transcriptional targets in RA are suggestive of a role for FoxO proteins in integrating inflammatory stimuli to maintain cell survival in RA synovial tissue. Active FoxO3α and FoxO4 can protect cells from oxidative stress through transcriptional up-regulation of the ROS (reactive oxygen species) scavenger MnSOD and GADD45 (growth-arrest and DNA-damage-inducible protein 45), a DNA repair protein [36,37]. Expression of both of these proteins decreased in RA synovial tissue or RA FLS compared with control tissues and cells [38]. Thus PI3K-dependent inactivation of FoxO proteins in RA FLS may sensitize these cells to the accumulation of oxidative stress-induced mutations in p53 and other tumour suppressor genes, allowing FLS to persist and proliferate in RA synovial tissue [39]. Another FoxO target which regulates cell cycle, p27 [40], is up-regulated in the synovial tissue of patients with active RA, as compared with patients in remission [41].

Elevated expression of the Bcl-2 family member Bcl-Xl has been proposed to protect T-cells from apoptosis in RA synovial tissue [42]. The ability of CD28 and cytokines to maintain synovial T-cell viability ex vivo may depend in part on its capacity to up-regulate Bcl-Xl, via inactivation of FoxO proteins as well as mTOR-mediated translational regulation [43–46]. Expression of a pro-apoptotic Bcl-2 family member, PUMA (p53 up-regulated modulator of apoptosis), is stimulated by FoxO4 proteins, independently of p53 [47]. PUMA is also expressed in RA FLS independently of p53, and in RA synovial tissue PUMA expression is lowest in the intimal lining layer [48], where PKB activity is highest.

### Integration of PI3K and inflammatory signals by FoxO proteins

PI3K signalling is unlikely to act alone in modulating FoxO transcriptional activity in RA. A series of recent studies has provided evidence that stress signals, including oxidative stress and TNFα stimulation, oppose or modify PKB-dependent inactivation of FoxO proteins [49]. ROS and oxidative stress play a central role in the pathogenesis of RA [50]. Cells in inflamed joints are exposed to ROS via repeated ischaemia/reperfusion, proximity to activated neutrophils and macrophages, the release of iron cations from dying cells, and stimulation by inflammatory cytokines that generate transient intracellular ROS production. Additionally, altered Ras family signalling in RA synovial T-cells exposes these cells to oxidative stress from endogenous ROS production [51]. ROS-dependent activation of Ste20-like kinases and JNK (c-Jun N-terminal kinase), the latter of which is highly activated in RA synovial tissue and is essential for induction of disease in animal arthritis models, phosphorylates FoxO proteins on residues distinct from PKB, promoting FoxO nuclear import and enhancing transcriptional activity [52–55]. Oxidative stress also stimulates FoxO nuclear association with and acetylation by p300/CPB [CREB (cAMP-response element)-binding protein] and p300/CPB-associated factor. Although acetylation reduces FoxO transcriptional activity, if phosphorylated by JNK, acetylated FoxO complexes with and is deacetylated by SIRT1 (sirtuin 1) and/or HDACs (histone deacetylases). Together, these activities appear to induce FoxO-dependent transcription of cell-cycle inhibitors and stress survival genes, while suppressing FoxO induction of apoptosis genes [49].

Might oxidative stress-dependent modification of FoxO function contribute to cell survival in RA? Tantalizing, albeit indirect, evidence consistent with this possibility has been published recently: Trichostatin A, an HDAC inhibitor, sensitizes RA FLS to TRAIL-induced apoptosis in vitro [56]. Additionally, HDAC inhibitors effectively protect rodents against autoantibody and adjuvant-induced arthritis [57,58]. Both in vitro and in vivo, the effects of HDAC inhibitors coincide with up-regulation of p21\(^{WAF1/Cip1}\), a FoxO transcriptional target [59].

### Concluding remarks

Further analysis of the activation status of intracellular signalling pathways in RA synovial tissue, in conjunction with analysis of gene expression profiles in RA patients, may identify molecular networks that differentially support inflammation and joint destruction, or predict therapeutic responses, in distinct RA patient subpopulations.

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### References


