Signaling Pathways Involved in Rheumatoid Arthritis: Targets for New Therapeutic Interventions

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ABSTRACT

Rheumatoid arthritis is categorized as a systematic autoimmune disease which causes chronic disabilities exclusively in bones that are aligned with synovium. RA aetiology is still unknown but previous studies have coined that several number of factors play a significant role e.g. environmental and genetic factors. Cellular signalling pathways orchestrate the inflammatory response that regulates various cellular functions like cellular progression, proliferation, death and secretion of signalling molecules (pro and anti-inflammatory cytokines) in response to genetic and environmental stimuli. These regulatory pathways are tightly controlled and naturally activated by ligands that attach to their respective receptors on the cell surface. In diseased state, these signalling pathways escape the normal control mechanisms, resulting in intensification of cytokines and chemokines, transcription factors and mediatory proteins that disrupt normal cell processes and might bring about auto-destructive consequences such as in the case of rheumatoid arthritis. The review highlights multiple levels of targeting molecules in signalling pathways that may be potential diagnostic markers and also attempts to underline potential therapeutic targets.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by inflammation of joints especially small joints of hands and feet ultimately leading to joints deformation and bone destruction. It involves imbalances in pro-inflammatory and anti-inflammatory cytokines levels (Paunovic & Harnett, 2013). RA develops as a result of combination of genetic and environmental factors. About 1% of the adults all over the world are reported to be affected by RA. Women are 2-3 times more prone to this disease as compared to men (Tobon, Youinou et al 2010) A well-defined twin study showed the involvement of genetic factors in RA accounting for 15-30% concordance rate in case of monozygotic twins and 5% in case of dizygotic twins (McInnes & Schett, 2011).

RA is best characterized by elevation in the levels of proinflammatory cytokines leading to persistent inflammation and subsequent damage to bone and cartilage. The signalling pathways of these inflammatory cytokines have emerged to have promising potential to be targeted by well-defined therapies thereby inhibiting inflammation and disease pathogenesis (Schett, 2011).

Depending on the progressive autoimmune nature, certain complexities are linked within a number of organ systems, which worsens the condition. The basis for such chronic inflammatory processes are sustained communication networks between cells within a tissue type and between different tissues. Signalling pathways lead to inflammation and various other cellular processes comprising of apoptosis, cell proliferation and cell differentiation. The signalling cascades when escape the balanced mechanisms, lead to disease pathogenesis or cellular destruction as observed in systemic rheumatoid arthritis (Tak, 2009).

This review highlights various signalling pathways involved in pathogenesis of RA e.g. MAPK pathway, Wnt signalling pathway, JAK/STAT pathway, NFκB signalling and TLR signalling, their activation, function, role in regulating cytokines and promoting inflammation and subsequent tissue destruction and also aims to summarize potential therapeutic targets identified so far in order to provide an efficient therapy for the disease.

1.1. MAPK Signalling in Rheumatoid Arthritis

Cellular responses are assimilated to environmental stresses by a group of signal transducing enzymes known as mitogen-activated protein kinases abbreviated as MAPKs(Inoue et al., 2006). MAPKs play important role in B / T Cell Receptor (BCR/TCR) signaling and also involved in signaling via other receptors such as toll-like receptors (TLRs), Interleukin Receptors including IL-1R, IL-17R and TNF-α. MAPKs are also regarded as important regulators involved in production of proinflammatory cytokines including TNF-α, IL-12, IL-23, IL-1, and IL-6 (Harnett et al.,2005; kolls and Linden, 2004; Lubberts et al.,2005). MAPKs consist of a highly conserved family of serine/threonine kinases involved in the regulation of important cellular processes including survival and cell growth, programmed cell death or apoptosis, proliferation/division, differentiation/specialization and inflammations in response to different stress molecules (Thalhamer et al., 2008). In mammals, three major types of MAPKs are reported namely, Extracellular signal Regulated Kinases (ERKs), C-Jun N-terminal kinase (JNK) and p38 MAPKs. JNK and p38 MAPKs collectively forms a family of kinases named as Stress Activated Protein Kinases (SAPKs)

(Thalhamer et al., 2008).

A signaling cascade, highly conserved among the species, leads to the activation of MAPKs (Dong et al., 2002; Johnson and Lapadat, 2002). First, MAPK Kinase Kinases (MAPKKK or MKKK) are activated that leads to activation of MAPK kinases (MAPKK or MKK) that ultimately leads to the activation of MAPKs in a complex signaling cascade (Figure 1A).MAPKKKs are serine/threonine protein kinases that activates MAPKKs through phosphorylation, while MAPKKs are protein kinases involved in activation of MAPKs through threonine and tyrosine phosphorylation in its highly conserved Threonine – X – Tyrosine motif (Thalhamer et al., 2008). The X residue in the motif is different for each of the MAPK class such as ERK contain Threonine - Glutamic acid – Tyrosine motif, Threonine – Proline -- Tyrosine motif in JNK while Threonine – Glycine – Tyrosine motif in p38 MAPKs. T–X–Y motif phosphorylation leads to MAPKs activation following downstream phosphorylation/activation of different proteins, for instance transcription factors. X residue in T—X—Y motif determines substrate specificity for MAPKs (Dong et al.,2002; Johnson and Lapadat, 2002). Different MAPKKKs and MAPKKs having unique specificity for substrates and activation kinetics can activate a certain MAPKs that allows for activation of a single MAPK through a variety of different stimuli, antigens and co-stimulatory receptors including certain cytokine receptors, FcRs and TLRs (Harnett et al.,2005; Ropert, 2005; Sweenay and Firestein, 2006).

MAPK activation is regulated through negative feedback mechanisms. For instance, not less than three classes of protein phosphatases, for instance, tyrosine phosphatases, threonine phosphatases and dual-specificity phosphatases, are induced by MAPKs that inhibit MAPKs through dephosphorylation (Jeffrey et al., 2007; Owens and Keyse, 2007). Specifically HePTP that is a tyrosine phosphatase, PP2A that is a threonine phosphatase and MKP3 that is a dual specificity phosphatase are the three MAPK phosphatases for ERK2 (Zhou et al., 2002). The dual-specificity phosphatases are a family of protein that can dephosphorylate the T–X–Y motif at both threonine and tyrosine residues leading to its inhibition (Camps et al., 2000). The dual-specificity phosphatases are regulated at transcriptional level and targeted inhibition of MAPKs is due to expression of protein phosphatases having affinity for specific substrate (Saxena and Mustelin, 2000). Procaspase activating compound (PAC-1) regulate ERK and p38 through dephosphorylation while MKP-1 is found to inhibit all types of MAPKs through negative feedback mechanisms(Thalhamer et al., 2008).

Dysregulated activation of MAPKs is linked to pathogenesis of Rheumatoid Arthritis in several studies(Paunovic and Harnett, 2013). Synovial inflammation, progressive cartilage destruction, bone erosion and angiogenesis are characteristic features of Rheumatoid arthritis and MAPKs are found guilty of involvement in each of these stages of disease (Figure 1B). Synovial tissue analysis of Rheumatoid Arthritis (RA) and osteoarthritis patients revealed MAPKs presence. Evidence of phosphorylated i.e. activated MAPKs in tissues from RA patients further established its role in the disease pathogenesis (Schett et al., 2000).

Table 1. Roles of various Signaling pathways in RA and potential therapeutic targets

Signaling Pathway	*Normal Role*	*Role In RA*	*Therapeutic Targets*	*References*
MAPK Signaling	• BCR/TCR signaling; • Survival and growth; • Apoptosis; • Proliferation; • Differentiation; • Inflammation.	• Synovitis; • Bone erosion; • Cartilage damage	• P38 MAPKs; • JNKs; • ERKs	Thalhammer*et al*., 2008 ; Li *et al*., 2013; Paunovic and Harnett, 2013
OG! Signaling	Mediating innate and adaptive immunity; Limiting inflammation; Cell proliferation	nduces expression of proinflammatory genes roduction of matrix metaloproteinases from synovial fibroblasts ecruits immune cells to the inflamed pannus	roteasomal inhibition to prevent NFkB activation nhibition of IKKs	Lawrence, 2009; Simmonds and Foxwell 2008
TLR Signaling	• Inflammation by activating NF-kB, and chemokines (TNF-, , IL-1 , IL-6) against PAMPs and DAMPs	• Synovial inflammation mediated by activating TNF-, , IL-8, IL-6, IL-15; • Destruction of bone by causing differentiation of monocytes into osteoclasts; • Increased risk of cardiovascular complications by activating MIF; • Maintenance of inflammation by expression of angiogenic factors VEGF, IL-8.	• Inhibition of adaptors (MyD88 and MAL/TRIP) to reduce cytokine and MMPs Expression; • Targeting IL-15; • Targeting MIF (macrophage migration inhibiting factor).	Ospelt*et al*., 2008; Sacre*et al*., 2007; Jung *et al*., 2007; Kim *et al*., 2007; Popa*et al*., 2006; Cho *et al*., 2007
Wnt Signaling	ell polarity; ell migration; one metabolism; ynovium proliferation, and rganogenesis	LS activation; timulate proinflammatory cytokines, and annus formation	Wnt1, Wnt5a, Wnt7b, z5 receptor, RANKL	Katoh and katoh, 2007; Miller *et al*., 1999
JAK-STAT Signaling	• Transcriptional regulation in reaction to binding of extracellular signaling molecule (cytokine, growth factors, chemokines) with the transmembrane receptor	• Constitutive STAT-3 DNA binding activity possibly caused by IL-6 leads to abnormal gene expression which is consistent with inflammation and active immunity	• Controlling Jak3 activity, inhibition of activated STAT-3; • inhibiting Jak-STAT signaling by induction of suppressors of cytokine signaling (SOCS) proteins	Darnell, Kerr, & Stark, 1994; van der Pouw Kraan *et al*., 2003; O'Shea, Park, Pesu, Borie, &Changelian, 2005; Ivashkiv& Hu, 2003

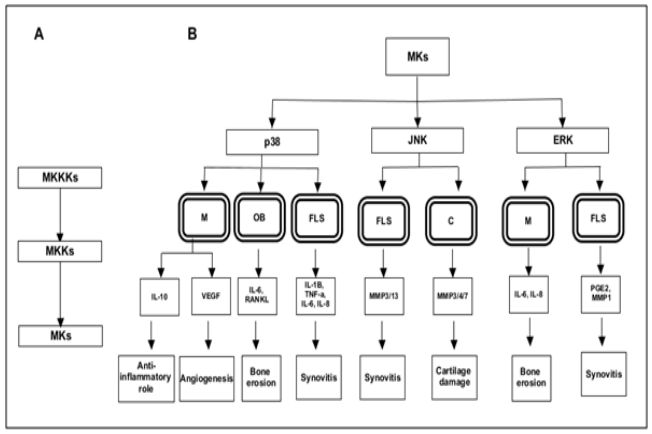

Figure 1. A. Activation cascade of MAPKs. **B.** Role of MAPKs in RA pathogenesis. MKKKs MAPK kinase kinases, MKKs MAPK kinases, MKs Mitogen activated protein kinases (MAPKs), JNK c-Jun N-terminal Kinases, ERK extracellular signal-regulated kinases, M macrophage, OB osteoblasts, FLS fibroblast like synoviocytes, C chondrocytes, IL-1B/6/8/10 interleukins, VEGF vascular endothelial growth factor, RANKL receptor activator of nuclear factor kappa B ligand, TNF-α tumour necrosis factor, MMP 1/3/4/7/13 matrix metalloproteinases, PGE2 prostaglandin E2.

Phospho-p38 MAPK is reported in synovial microvessel endothelium as well as in the synovial layer's lining. While phosphorylated and activated ERK-MAPK was found in cells residing in synovial lining and mononuclear infiltrates. Whereas activated JNK-MAPKs were found to be resident of sub lining mononuclear cell infiltrates (Schett et al., 2000). IL-6, TNF-αand IL-1 induce IL-8, Matrix Metalloproteinases and various adhesions molecules production and thus accused to be involved promoting RA in the synovium through enhancement of cell infiltration, inflammatory responses and destruction of cartilage(Thalhamer et al., 2008). IL-6, TNF-α and IL-1 activates all classes of MAPKs in synovial fibroblasts (Schett et al., 2000) while in chondrocytes, ERK, JNK and p38 MAPK were found to be activated by IL-1 and TNF-α leading to induction of Matrix Metalloproteinases including MMP-1 and MMP-13(Masuko-Hongo et al., 2004; Liacini et al., 2003; Mengshol et al., 2004). Moreover, in inflamed synovial tissue IL-1 and TNFα are reported to have a vital role in p38 and ERK-MAPK activation in vivo, as their inhibition lowered the MAPKs activation in transgenic mice expressing TNF-α (Gortz et al., 2005). Furthermore, role of these feedback mechanisms of MAPK signailing was emphasized by studies on murine, in which collagen induced arthritis abbreviated as CIA was worsen due to MAPK phosphatase-1 (MKP-1) deficiency (Salojin et al.,2006). However, reduced inflammatory responses were observed in PAC-1 (dual-specificity phosphatase) knockout mice. Further findings indicated that all MAPK classes are erroneously activated in synovial tissues and their differential activation patterns may be a sign of their involvement in the disease pathogenesis(Thalhamer et al., 2008).

All of the above mentioned reports establishes MAPKs as a potential molecular targets for therapeutic intervention for rheumatoid arthritis (Paunovic and Harnett, 2013; Li et al., 2013). In order to create safe and effective drugs, MAPKs Inhibitors are of recent research interest (Li et al., 2013). In the light of recent findings and analysis, p38 -MAPK is established as a key molecular target for an anti–proinflammatory cytokine production leading to therapy for Rheumatoid Arthritis. The α-isoform of p38 is expressed in the RA synovium and is involved in regulation of intracellular pathways of TNF-α, IL-1β, and cyclooxygenase 2 production (Malemud and Miller, 2008; Thalhamer et al., 2008). Inhibitors of p38α are reported to successfully suppress the production of TNF-α and IL-1β in monocytes and arthritis animal models (Nishikawa et al., 2003). Therefore, it can evaluated that compounds inhibiting p38 MAPK activation may have beneficial therapeutic use in autoimmune disorders including RA. Despite a speedy progress on p38 MAPK inhibitors, little evidence exists claiming it to be an effective approach in RA. An extensive research is ongoing in this area studying p38-MAPK inhibitors in RA and in other chronic inflammatory diseases are either incomplete or are in progress (Cheriyan et al., 2011; Zhang et al., 2010).

1.2. NFκB Signalling in Rheumatoid Arthritis

NFkB constitutes a family of ubiquitously expressed transcription factors which are significant regulators of inflammation and various immune responses (Abu-Amer and Faccio, 2006). NFkB is essential for cell proliferation and signal transduction for normal vertebrate development. There are five types of NFkB TFs found in mammals which include RelA (p65), RelB, c-Rel, NFkB1 (p50/p51 precursor) and NFkB2 (p52/p100 precursor). When active, different members of NFkB form different homodimers and heterodimers of any of its five subunits each activating a particular gene set. The most prevalent active heterodimer is RelA (p65) and p50 (J.A. Roman-Blas and S.A. Jimenez, 2006). A stretch of 300 amino acids known as homology domain is present in all five members of NFkB protein family that mediates dimerization, nuclear translocation of NFkB and its association with DNA (Ghosh and Karin, 2002; Carlsenet al., 2004; Bouwmeesteret al., 2004). Binding of NFkB TFs to DNA occurs at specific promoter regions known as Rel sites (Karin et al., 2004).

NFkB is primarily present in the cell cytoplasm sequestered by inhibitory kB proteins (IkB) which render it inactive (Figure 2). IkB proteins include IkBα, IKBβ, IkBγ, IkBε (Li and Verma, 2002; Ghosh and Karin, 2002).For binding to NFkB proteins, IkB proteins contain ankyrin repeats at the C-terminal. While at the N-terminal, it contains a nuclear export signal thus, controlling the shuttling of NFkB between nucleus and cytoplasm (Roman-Blas & Jimenez, 2006). Binding with IkB is crucial to retain p50-p65 heterodimer within the cytoplasm.

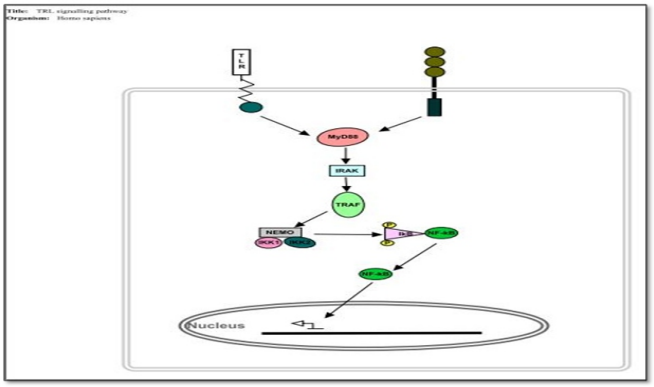

Figure 2. Upon stimulation, TLR/IL-1R initiate downstream signalling via MyD88 activating IRAK, TRAF and IKKs. IKK1 and IKK2 phosphorylate IkB leading to its proteolytic degradation and allowing translocation of NFkB into the nucleus. NFkB regulates transcription of genes involved in inflammation.

A wide range of stimuli including TNFα, IL-1, UV radiations, free radicals, bacterial and viral products which activate TNF receptors, IL-1 receptors/TLR superfamily, Nod-like receptors, B cell and T cell receptors (O'Neill, 2006) result in phosphorylation and subsequent degradation of IkB by E3 ubiquitin ligase complex and 26S proteasome. Phosphorylation of IkB inhibitors at serine/threonine residues is carried out by IkB kinases (IKK) (Roman-Blas & Jimenez, 2006) which comprise IKKα (IKK1) and IKKβ (IKK2). IkB Kinases are therefore key regulators of NFkB activity. NFkB essential modulator (NEMO), a scaffold protein, is another important regulator of NFkB activity.

NFkB signaling pathway plays multiple important functions for organismal growth and development. However, when normal functioning goes array, irregular NFkB signaling gives rise to diseases like Rheumatoid Arthritis, osteoarthritis (OA), asthma, inflammatory bowel disease, Diabetes Type 2 and cancer. p50 and p65 have been found to be abundant in RA and OA synovitis (Handel et al., 1995). Synovial tissues of RA patients have a higher number of cells producing NFkB1 at the cartilage-pannus junction than other body cells (Benito et al., 2004).

IKK2 activates the NFkB canonical pathway whereas IKK1 activates the non-canonical pathway by phosphorylating p100 and activating p52 (J.A. Roman-Blas and S.A. Jimenez, 2006). Despite of having structural similarity with IKK1, IKK2 has been found to play a dominant part in NFkB activation when fibroblast-like synoviocytes (FLS) are stimulated with cytokines (Udalova et al., 2002). Activation of IKK2 is the key to NFkB-mediated synthesis of IL-8, IL-6, and intercellular adhesion molecule 1 (ICAM-1) after cell stimulation with TNFα and IL-1 occurs (Aupperle et al., 2001). It has been shown that when a dominant negative IKK2 adenoviral construct is used to block IKK2 in vitro, synthesis of IL-1, IL-6, IL-8 and ICAM-1 doesn't occur after FLS treatment with cytokines. However, in Rheumatoid Arthritis production of TNFα is not dependent upon IKK2 (Andreakos et al., 2003).

RA is a chronic inflammatory joint disease in which synoviocytes play a significant role in causing cartilage damage by producing IL-6 and IL-8. A study showed that treatment with simvastatin inhibited IL-6 and IL-8 production from IL-1 stimulated FLS by inactivating the NFkB pathway (Lazzerini et al., 2007).

Upon reception of an appropriate stimuli, the TNF receptors recruit TNF receptor-associated death domain (TRADD), receptor-interacting protein 1, TNF receptor-associated factor 2 (TRAF2). IL-1/TLR family initiates signal transduction via MyD88, MyD88 adaptor-like (MAL), Toll/IL-1 receptor domain-containing adaptor protein inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM) (O'Neill, 2006). Likewise, T cell and B cell receptors also recruit a number of signalling molecules in order to activate NFkB pathway. All these complex pathways present a challenge to develop an effective inhibitor of NFkB activation. Many selective inhibitors of IKKs and NEMO have been developed which show promise for future RA therapies.

1.3. TLR Signalling in Rheumatoid Arthritis

Toll-like receptors (TLRs) are evolutionary conserved receptors expressed on various cell types including myeloid cells, fibroblasts, epithelial cells etc. They recognize certain molecules associated with foreign beings (PAMPs) and generate response. TLR4 recognize bacterial lipopolysaccharide (LPS) using adaptor proteins MyD88, CD14 and LBP, TLR1, 2 and 6 recognize lipoproteins, TLR5 recognizes flagellin, TLR9 sense the CpG island of bacterial DNA while TLR3, 7 and 8 bind to viral DNA/RNA (Goh et al., 2012). There has also been evidence of DAMPs (damage-associated molecular patterns) i.e. molecules released by necrotic cells e.g. systolic heat-shock proteins and extracellular matrix components like fibrinogen, tenascin-C, heparin sulphate, hyaluron (HA) all of which are up-regulated in response to damage are recognized by TLRs (Goh et al., 2012).

All TLRs are transmembrane receptors which use leucine rich repeats in the extracellular domain to recognize ligands while the cytoplasmic domain has a conserved motif (Toll/interleukin (IL)-1 receptor (TIR) domain) this domain interacts with the adaptor proteins to trigger downstream signalling (Brentano et al., 2005).

Once activated by the PAMPs or DAMPs downstream signalling is brought about by various adaptor proteins; MyD88, MAL/TIRAP, TRIF, TRAM and SARM. All TLRs signal via MyD88 except TLR3 which utilizes TRIF (Santegoets et al., 2011). These adaptors activate protein kinases e.g. MAPK leading to activation of various transcription factors like AP-1, NF-kB all of which transcribe the production of inflammatory cytokines and chemokines TNF-α, IL-1β, IL-6 and interferons (Santegoets et al., 2011).

The signalling pathway can be MyD88 dependent or independent. In the MyD88 dependent pathway, the adaptor protein mediates the phosphorylation of IRAK 1 and 4 which then bind to TRAF6 activating IKK complex thereby inducing the activation of NF-kB transcription factor and MAP kinases (Brentano et al., 2005). While the hallmark of independent pathway involves the activation of transcription

factor IRF-3 consequently leading to the induction of IFN expression which involves TRIF adaptor (Brentano et al., 2005).

In rheumatoid arthritis (RA) number of studies has given evidence of the role of TLRs in the pathogenesis of the disease thereby also providing therapeutic targets for treatment. Ospelt et al (2008) determined levels of TLRs (1-10) in RA as compared to normal. TLR3 was expressed most followed by TLR4 then TLR2 while TLR10 was not detected in tissue and fibroblasts (Ospelt et al., 2008). It can be deduced that activation of TLRs occurs early leading to synovial inflammation and joint destruction. While another study by Roelofs et al (2005) showed that TLR3 and 7 were expressed in synovium in RA patients and stimulation with their respective ligands led to dendritic cell maturation and production of IL-6 and TNF-α. Expression of various TLRs was also determined by Seibl et al (2003) to investigate their regulation in RA. In both synovial fibroblasts and tissue TLR2 expression is up-regulated after stimulation with its ligand as well as cytokines IL-1β and TNF-α along with the translocation of NF-kB to the nucleus (Seibl et al., 2003).

Similarly the role of TLRs in chronic inflammation was determined using four RA patients. TLR 2 and 4 was seen to be up-regulated while the addition of their respective ligands showed marked increase in TNF-α and IL-8 mediated by adaptors MyD88 and MAL/TIRAP as determined by respectively inhibiting those (Sacre et al., 2007). This provided evidence that the adaptors were needed for cytokine production, inhibiting the adaptors also significantly reduced expression matrix metalloproteinases (MMPs) (Sacre et al., 2007) which are involved in tissue destruction. It could thus be deduced that in addition to cytokine production TLRs also regulate the destructive processes via MMPs.

Since inflammatory cytokines leading to persistent synovial inflammation are characteristic of RA, the linkage between TLR signalling and the production of such cytokines has been studied in different studies. A particular group used stimulating ligands of TLR2 and 4 to determine expression of IL-15. IL-15 is upregulated both by TLR 2 and 4 which is brought about by downstream signalling via NF-kB (Jung et al., 2007). IL-15 is linked to inflammatory diseases, when present in synovium it activates T cells, neutrophils while also reducing apoptosis, promoting proinflammatory cytokines and autoreactivity. The initiation of IL-15 by stimulation with TLR2 and 4 might be important in pathogenesis of inflammatory synovitis and targeting IL-15 might be a potential target for treatment.

Destruction of bone and cartilage in RA is brought about by the action of cytokines utilizes the interaction of various cells (lymphocytes, macrophages) to activate MMPs and osteoclast activity. Osteoclasts are a class of macrophages that degrade bone matrix, their differentiation is directed by RANKL and M-CSF. In RA, osteoclastogenesis is increased causing bone resorption and destruction. RANKL expression was shown to increase when fibroblasts were stimulated with TLR2 and 4 ligands and also partly by the proinflammatory cytokines, TNF-α and IL-1β (Kim et al., 2007) which are products of TLR signalling. TRAP-positive cells which are markers for osteoclasts were also seen after monocytes were stimulated with TLR ligands (Kim et al., 2007). Involvement of TLR signalling in osteoclastogenesis was also shown by another study which suggested that TLR3 and RANKL expression was high in RA (Kim et al., 2009). This suggests that TLR2, 3 and 4 activation enhances expression of RANKL which assists osteoclastogenesis by causing osteoclast differentiation from monocytes. The system provides a direct link between inflammation and bone erosion.

MIF (macrophage migration inhibiting factor) plays a crucial part in pathogenesis of autoimmune disorder and are incriminated to increase the risk of cardiovascular morbidity by causing atherosclerotic plaques. It was shown that dendritic cells (DC) derived from RA patients had high levels of MIF after stimulation with TLR2 and 4 (Popa et al., 2006). This increased concentrations of MIF found in synovial fluid and serum can be used as a novel marker for the disease. The same study also tested the role of cytokines on MIF secretion, TNF- α and RANKL caused an increase in MIF (Popa et al., 2006). TNF-α secreted in RA induces the production of MIF which adds on to the destructive processes by the upregulation of matrix metalloproteinases (MMPs). The cytokines play a role in bone erosion and inflammation of joints thereby suggesting that by regulation of MIF is in fact a process to augment the inflammatory loop.

Another way to maintain synovitis and leukocyte influx in RA is by angiogenesis. Angiogenic mediators include IL-8, VEGF etc. The production of both IL-8 and VEGF were markedly increased when synovial fibroblasts were stimulated by TLR2 ligand (PGN) therefore IL-8, VEGF and TLR2 were co-expressed and resulting downstream signalling occurred via MyD88 dependent pathway (Cho et al., 2007). The synovium of RA patients is infiltrated by numerous cells at the site of inflammation and angiogenesis provide the route of transmigration therefore it plays a key role in mediating inflammation at early stages.

Pathogenesis of RA is complex with many components not all of which are elucidated. One pathway might include the dysregulation of unfolded protein response (UPR) to increased number of destructive synovial fibroblasts. One component of UPR is the XBP1 which is a transcription factor regulating genes involved in ER stress. TLR2 and 4 are implicated in splicing of XBP-1 in absence of ER stress and leading to production of inflammatory cytokines IL-6 and TNF while expression of spliced XBP-1 was seen in blood cells of patients with RA (Savic et al., 2014). However the full implications of this dysregulated pathway in RA disease pathogenesis still needs to be understood fully.

Evidence from the studies on animal models and cell cultures highlight the role of TLRs in RA pathogenesis, targeting these receptors and their signalling pathway might provide an effective therapeutic tool for treatment. It is also interesting to consider the extent with which each TLR interacts to bring about the pathological changes. RA is a heterogeneous disease with unpredictable therapy response therefore, there is need of novel biomarkers to aid in personalized medicine for effective therapy.

1.4. Wnt Signalling in Rheumatoid Arthritis

Wnt signaling pathway acts as an antique and evolutionarily preserved pathway that switches basic features of neural imitating, cell polarity, cell immigration, cell fate determination and embryonic organogenesis escalation (Komiya & Habas, 2008; M. Sen, 2005). Wnts are secreted glycoprotein (Wnt1, Wnt2, Wnt2B, Wnt3, Wnt4, Wnt5A, Wnt5B, Wnt6, Wnt7A, Wnt8A, Wnt8B, Wnt9A, Wnt9B, Wnt10A, Wnt10B, Wnt11 and Wnt16) that bind with frizzled (Fz) receptors and may also necessitate Low-density lipoprotein receptor-related protein 5/6(LRP 5/6) receptor that is in case of canonical pathway (Habas &Dawid, 2005; Li et al., 2012).

Wnts glycoprotein and Fz receptor multifaceted activates many down signaling including non-canonical β-catenin-independent pathway (Wnt/Ca2+ pathways and Planar Cell Polarity (PCP)) or canonical and Wnt/β-catenin pathway (Yamaguchi, 2001). In Canonical pathway Wnt1, Wnt3a, Wnt8 and non-canonical pathway Wnt5a, Wnt11 most of the time enrolled as Wnt modulator (Figure 3)(Kühl, 2004). Wnt signaling is essential for tissue maintenance that why Wnt signaling involves in human genetic disorder and other diseases like absence of limbs (Niemannet al., 2004), cancer (especially Familial Adenomatous Polyposis), and autoimmune diseases like Rheumatoid arthritis, lupus erythematosus (Kim et al., 2010).

Here we focus Wnt pathway in the development of RA. Current studies have revealed Wnt signaling pathway implicate in the pathogenesis of RA. Beta-catenin is principal element in the instigation of canonical Wnt pathway. B-catenin express high altitude in RA patient and also up regulates FLS expression (Kuanget al., 2009). Similarly down signaling in non-canonical Wnt pathway ignition through activation of Ca+2 mediated enzymes calcineurin (CaCN), protein kinase C (PKC) and calmodulin/Ca2+ dependent kinase II (CamKII) (Sheldahl, Park, Malbon, & Moon, 1999).

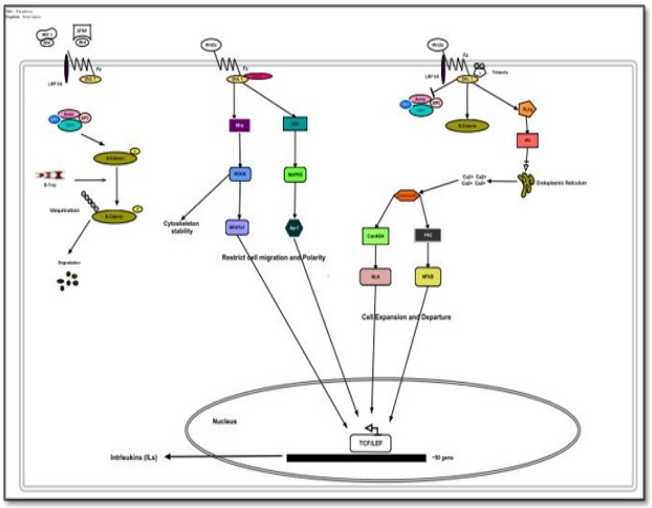

Figure 3. Wnt pathway mediated gene expression (left) canonical Wnt pathway that is mediated by LRP LDL related protein and activated by Wnt proteins. (middle) non-canonical Wnt pathway activation (left) Ca2+ dependent Wnt pathway that down signalling facilitated by IP3 inositol triphosphate, Ca2+, CamkIICa2+/Calmodulin-dependnet kinase II, NLK Ser/Thr-protein kinase, CaCN Calcineurin, result in activation of nuclear factors TCF/LEF T-cell specific transcript/lymphoid-enhancer binding factor.

Previous study confirmed that wnt5a express by FLS in RA patient. Wnt5a triggers PKC signaling cascade which further stimulate NFkB pathway, as a result stimulation of IL genes. IL genes are IL-15, IL-6 which are diagnostic marker in patient with chronic RA (Wilson, Szabo, & Salzman, 1999). So, IL-6 and IL-15 fabrication as result of Wnt-5/Fz5-mediated signaling by the RA FLS may cause pannus formation, cartilage destruction, and bone erosion. It has been reported that ani-Fz and anti-Wnt5a were blocking their activity(Aveleira, Lin, Abcouwer, Ambrósio, & Antonetti, 2010; Malini Sen, Chamorro, Reifert, Corr, & Carson, 2001). So, Wnt5a/Fz5 has potential as a therapeutic target. Wnt5a silencing through SiRNA technology also reduces the expression in RA patient (Katoh & Katoh, 2007).

RANKL expression was testified at vigorous bone erosions sites. Normal expression of RANKL in osteoblasts and stromal cells under the stress of hormones (calcitriol, PTH, prolactin, estrogens, glucocorticoids), growth factors (BMP, oncostatin M, TGFb, IGF1, PDGF) and cytokines (PGE , IL-1, IL-6, IL-17, TNFa) (Lorenzo, Horowitz, & Choi, 2008). RANKL production in RA is activated by synovial fibroblasts, macrophages, Th17 cells, activated B cells and dendritic cells (Okamoto & Takayanagi, 2011).Another approach to treat RA is overcome the expression of RANKL by using anti-Fz5. We can also obstruct osteoclastogenesis and bone erosion in RA pathogenesis (MaliniSenet al., 2001).

Wnt1-mediated signaling pathway show dynamic role in the development of RA. Wnt1-mediated signaling controlled the fibronectin manifestation in RA FLS through canonical Wnt signaling pathway. Wnt1-mediated signaling must endorse the articular cartilage impairment (Nakamura, Nawata, & Wakitani, 2005).

Wnt1 include significantly involved in Wnt7b elevated expression in the cartilage of osteoarthritis and synovium of RA patients. Wnt7b increased the pro-inflammatory cytokines expression IL-1B, TNF-α and IL-6 under FLS transfection. Wnt signaling pathway signifying that Wnt7b upstream factor also act as an imperative role in the pathogenesis of RA (Fouque-Aubertet al., 2012).

MiRNA validation assays and sequencing approve impairment of miR-146am miR-155 and miR-223 are related with RA patient, similarly other MiRNA that are miR-323-3p and miR-221/222. Bioinformatics analysis revealed that elevated level of miR-323-3p also associated to activation of Wnt pathway. miR-323-3p is used as diagnostic marker (Pandiset al., 2012).

1.5. The JAK-STAT Pathway in Rheumatoid Arthritis

Owing to the side effects caused by various immunosuppressive drugs being used in rheumatoid arthritis, research has been prompted in a different orientation (Walker & Smith, 2005). A molecule that is restricted to particular immune cells can serve as a target in designing immunosuppressive drugs with reduced toxicity. Protein

tyrosine kinase; Janus kinase (JAK) appeared as potential target. Cytokines have a central role in immune cell regulation (O'Shea, Pesu, Borie, & Changelian, 2004).

Cytokines belonging to the hematopoietin family bind to Type I and Type II cytokine receptors and initiate a signal cascade inside the cell by the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway. A number of such cytokines have been reported/are theorized to have a role in RA (e.g., interferons, IL-6, IL-2, IL-7, IL-12, IL-15) and therefore, a detailed understanding of JAK-STAT activation within the rheumatoid synovium will have a direct impact on the discovery and development of novel therapeutic agents for the disease (Walker & Smith, 2005).

JAKs are activated; auto and trans-phosphorylates by the cytokine binding with the receptor. These receptors are then phosphorylated by JAK to generate sites for docking of molecules that effect signaling. Signal transducers and activators of transcription (STATs) are the family of molecules that are significant for conducting cytokine signals and regulating gene expression (Darnell Jr, Kerr, & Stark, 1994).

Most of the JAKs bind several cytokine receptors and are widely expressed. JAK3 is specifically expressed, has narrow tissue distribution and interact particularly with one cytokine receptor subunit, making it a potential therapeutic target (Kawamura et al., 1994). Six cytokines bind to a common cytokine receptor subunit named γ-subunit (γc). The cytokines that selectively binds and hence activate JAK3 are: Interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21 (O'Shea, et al., 2004). Phosphorylated sites on cytokine receptors are identified by STAT and other molecules which are then recruited and activated by JAK driven tyrosine phosphorylation. STAT on activation disassociates from the receptor, form dimers in the cytoplasm which are then translocated to the nucleus where they bind to the γ -activated site (GAS) enhancers (Figure 4)(Walker & Smith, 2005).

Mononuclear cells isolated from RA synovial fluid (SF) showed integral STAT-3 DNA binding activity, and revealed that STAT-3 in control human monocytes was efficiently activated by the soluble factors present in SFs of RA patients (Sengupta, Chen, Zhong, Darnell, & Ivashkiv, 1995)(Wang, Sengupta, Zhong, & Ivashkiv, 1995). Immunohistochemistry was used to detect activation of tyrosine phosphorylation of STAT-3 in synovial tissue of RA patient in vivo. It is not certainly known that which cytokine is responsible for the activation of STAT-3 during synovial inflammation in RA in vivo, but IL-6 is the possible candidate (Ivashkiv & Hu, 2003).

STAT-3 activation and increased expression of STAT-1, STAT-4, and STAT-6 had been described in RA synovitis. (Fruchtet al., 2000)(Müller-Ladner et al., 2000). However, it was not sure whether these STATs were able to control gene expression and phenotype of synovial cell, in short can perform their function fully or not, in synovitis. STAT function can be assessed by the measurement of the STAT-dependent genes expression. Van der PouwKraan and coworkers used the same approach and used microarray technique to produce an ample profile of gene expression in RA synovium (van der Pouw Kraan et al., 2003). This significant study outspreads information obtained from former gene expression profiling experiments in RA, and illustrates two patterns showed by RA tissues. The first group showed gene expression regular with active immunity and inflammation, with significant expression of genes of antigen-presenting cell and lymphocytes, along with genes encoding transcription factors, activation markers, signaling molecules, chemokines, and cytokine receptors. The second group of RA tissues had gene expression profile more alike to osteoarthritis tissues. These tissues expressed genes imperative in tissue remodeling and showed less expression of immune and inflammatory genes (Ivashkiv & Hu, 2003)(van der Pouw Kraan, et al., 2003).

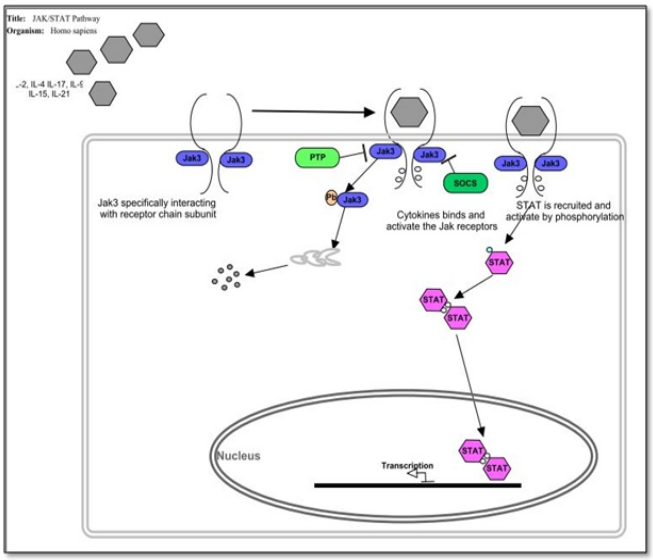

Figure 4. JAK-STAT pathway in Rheumatoid arthritis

These anomalies in gene expression in the diseased state may either be variant in various classes of RA, or specific to different stages in disease course, characterized by varied activity and ultimately leading to rigorously damaged joints with reduced inflammation in a final "burned out" stage (Ivashkiv & Hu, 2003)(van der Pouw Kraan, et al., 2003).

Closely related DNA sequences are recognized by the different STATs (Ivashkiv, 1995), and it has been shown (Nakajima et al., 1996) that STAT-3 can trigger expression of some of the "STAT-1–dependent genes". STAT-3 is essentially activated in RA synovium which further supports the concept of a likely role for STAT-3 in activation of STAT-dependent genes in these tissues. Also there is indication that "STAT-1 dependent genes" can be triggered to be expressed in a STAT-3–dependent way by IL-6, along with an unidentified SF factor. (Sengupta, et al., 1995).

As JAK3 is selectively expressed in specific tissues, it was suggested that interrupting with Jak3 function could be the basis for a novel class of immunosuppressants. Furthermore, because the Jak3 results in immunodeficiency but not pleiotropic defects, an exceedingly specific Jak3 inhibitor should also have precise restricted and specific effects. JAK3 targeting contrasts with broadly used immunosuppressive drug, which shows non-particular targeting and have varied

side effects. In standard, Jak3 inhibitor selection would have benefits over the recent agents (O'Shea et al., 2005).

Another strategy includes initiation of suppressors of cytokine signaling (SOCS) proteins which fade experimental arthritis. This illustrates the role of STATs in pathogenesis of rheumatoid arthritis (Ivashkiv and Hu, 2003). Inhibition of JAK-STAT signaling by SOCS family members can be done by inhibiting catalytic activity of JAK, consequently inhibiting receptor docking sites for STAT and targeting cytokine receptors for deprivation by proteasomes (Walker and Smith, 2005). Therefore SOCS can assist as drug against rheumatoid arthritis via inhibiting STAT activity and ultimately RA pathogenesis

2. CONCLUSION

Various important signalling pathways when become aberrant, as described in this review, could manifest disease pathogenesis as it occurs in Rheumatoid Arthritis (Table 1). Disarrayed signalling pathways present a possible target therapy for RA. Blocking signal transduction by using gene therapy, peptide inhibitors and small interfering RNA techniques to target important signalling components is being exploited for the treatment RA. However, as these signalling molecules are involved in various other overlapping pathways important for metabolic function, a more plausible option is prevention of receptor activation to block a specific pathway. For the development of such inhibitors there are still a number of challenges to overcome in order to develop effective therapeutic drugs for RA.

Conflict of Interest

All authors have no conflict of interest regarding this manuscript for publication.

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