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REVIEW ARTICLE

Takashi Okamoto

The epigenetic alteration of synovial cell gene expression in rheumatoid arthritis and the roles of nuclear factor κB and Notch signaling pathways

Abstract Rheumatoid arthritis (RA) is a complex process of chronic and progressive inflammation associated with activation of numerous signaling molecules and transcription factors and hyperproliferation of synoviocytes of the affected joints, although the greater part of its pathophysiological process is explained by activation of nuclear factor κB (NF-κB). For example, the self-perpetuating nature of the rheumatoid inflammation is ascribable to overexpression of the proinflammatory cytokines tumor necrosis factor α and interleukin-1β, known to elicit the activation cascade for NF-κB and activator protein-1 that are responsible for transcriptional induction of these cytokines among other target genes, which conform a positive feedback loop for continuation and expansion of the inflammatory responses. In addition, comparative gene expression profile analyses have revealed activation of a number of genes that explain the "transformed-like" phenotype of synoviocytes. Among the genes expressed in rheumatoid synoviocytes upon inflammatory stimuli, induction of gene expression of Notch proteins and its ligand have been found. Possible roles of Notch signaling in RA synoviocytes are discussed.

Key words Notch · Nuclear factor κB (NF-κB) · Rheumatoid arthritis (RA) · Signal transduction · Synoviocyte

Introduction

Rheumatoid arthritis (RA) is a common human autoimmune disease with a prevalence of about 1%.[1] While there has been progress in defining its etiology and pathogenesis, these are still incompletely understood.[1–3] Proposed causes for RA include (i) genetic predisposition, (ii) pathogenetic immunoinflammatory responses triggered by environmental agents, particularly microbes, (iii) autoimmunity directed against components of synovium and cartilage, (iv) dysregulated production of cytokines (usually upregulation of proinflammatory and inflammatory cytokines and chemokines), (v) recruitment of immunoinflammatory cells through induction of inflammatory cell adhesion molecules (such as E-selectin, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1), and, last but not least, (vi) transformation of synovial cells into autonomously proliferating cells with highly invasive nature (often referred to as "transformed-like" phenotype[4–6]).

Rheumatoid arthritis is characterized by a chronic inflammation of the synovial joints associated with proliferation of synovial cells and infiltration of activated immunoinflammatory cells including memory T cells, macrophages, and plasma cells,[1,2,7] which eventually leads to progressive destruction of cartilage and bone. This process is considered to be mediated by a number of cytokines including tumor necrosis factor α(TNFα), interleukin (IL)-1, IL-6, IL-8, IL-12, IL-16, IL-18, and interferon γ(IFNγ) (reviewed in Refs. 1–3). Most of these pathophysiological features of RA can be explained by activation of limited number of transcription factor and its activation signals such as nuclear factor κB (NF-κB) and activator protein (AP)-1.[3,8] In fact, some effective anti-RA drugs are now known to inhibit NF-κB and its activation cascade (reviewed in Ref. 8). However, the mechanism by which rheumatoid synoviocytes exhibit the tumor-like nature has been yet to be clarified.

Involvement of NF-κB in RA as a primary pathogenic determinant

Among the various signaling and transcription regulation pathways, NF-κB and AP-1 are known to be the target of inflammatory responses. In fact, most of the factors involved in RA pathophysiology are under the control of these transcription factors.[3,8] Particularly, various cytokines

T. Okamoto (✉)
Department of Molecular and Cellular Biology, Nagoya City University Graduate School of Medical Science, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan
Tel. +81-52-853-8205; Fax +81-52-859-1235
e-mail: tokamoto@med.nagoya-cu.ac.jp

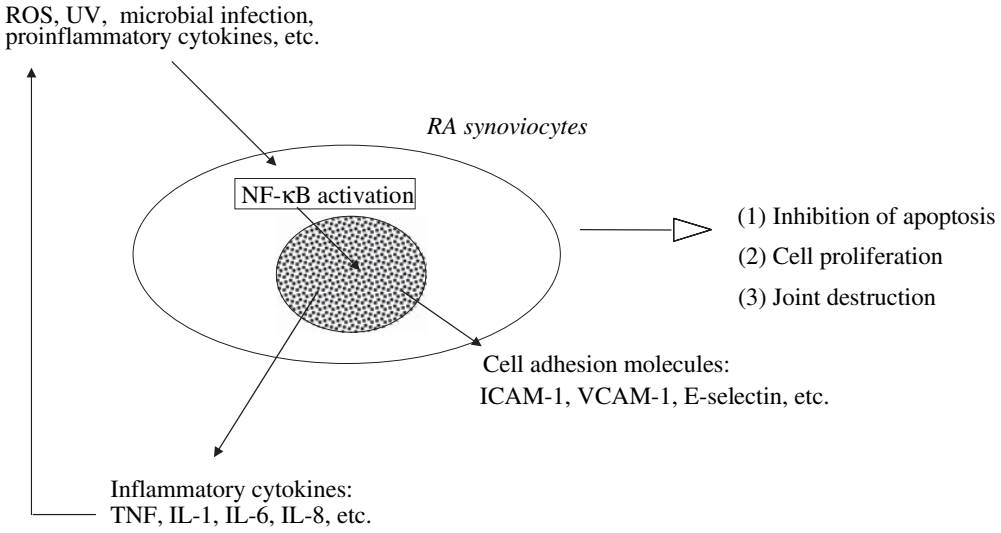

Fig. 1. Involvement of nuclear factor κB (*NF-κB*) in rheumatoid arthritis (*RA*) pathophysiology. NF-κB induces gene expression of inflammatory mediators such as cytokines and cell adhesion molecules. Since proinflammatory cytokines, tumor necrosis factor α (*TNF*), and interleukin (*IL*)-1β stimulate the NF-κB activation cascade that induces expression of these cytokines, there will be a positive feedback loop that perpetuates and expands the inflammatory responses even systemically. NF-κB also stimulates synovial proliferation by inhibiting apoptosis. See also Fig. 2. *ROS*, reactive oxygen species; *ICAM*, intracellular adhesion molecule; *VCAM*, vascular cellular adhesion molecule

and cell adhesion molecules activated in the rheumatoid joints are under the transcriptional control of NF-κB. The self-perpetuating nature of rheumatoid inflammation is ascribable to TNFα and IL-1β, known to elicit the activation cascade for NF-κB and AP-1, as they constitute another positive feedback loop in the logic of the inflammatory responses associated with RA (Fig. 1).

In addition, besides its action in upregulating inflammatory cytokines and cell adhesion molecules, NF-κB also induces gene expression of cell growth-promoting factors such as cyclin D1 and c-Myc, and physiological inhibitors of apoptosis such as cIAPs, Bcl-X_L, and cFLIP.[9,10] (Fig. 2). Moreover, it has been shown that NF-κB blocks apoptosis in the absence of de novo protein synthesis[11] through protein–protein interaction with p53 and proapoptotic protein 53BP2.[12,13] These actions of NF-κB explain not only the inflammatory responses but also the hyperproliferation of synovial tissues in RA, indicating that NF-κB acts as a major determinant for RA pathophysiology. Nuclear factor κB induces TNFα and IL-1β gene expression, and both TNFα and IL-1β stimulate NF-κB signaling, a vicious cycle formed to perpetuate and even expand the inflammatory responses.[8] The intervention therapy using anti-TNF antibody and IL-1β receptor antagonist has been thus developed.[14,15] In addition, some of the drugs for RA have been shown to block NF-κB-activation cascade or its actions (Table 1).[16–18]

The signal transduction cascade for NF-κB activation

The members of the NF-κB family in mammalian cells include the proto-oncogene c-Rel, Rel A (p65), Rel B, NFkB1 (p50/105), and NFkB2 (p52/p100). These proteins share a conserved 300-amino-acid region known as the Rel homology domain, which is responsible for DNA binding, dimerization, and nuclear translocation of NF-κB. In most cells, Rel family members form hetero- and homodimers with distinct specificities in various combinations.[8,19,20] A common feature of the regulation of NF-κB family is their sequestration in the cytoplasm as inactive complexes with a class of inhibitory molecules known as IκBs.[20,21] Upon stimulation of the cells such as by proinflammatory cytokines, IL-1β and TNFα, IκBs are degraded, and NF-κB is translocated to the nucleus and activates expression of target genes (Fig. 2).

The IκB kinase complex capable of specifically phosphorylating Ser32 and Ser36 of IκBα was originally identified as a ~700 kDa of high molecular complex.[21,22] Subsequently, two catalytic subunits (IKKα and IKKβ) and a scaffold subunit of this complex (IKKγ/NEMO/IKKAP) were identified and cloned (for review see Refs. 20–22). The IKK complex, consisting of IKKα, β, and γ, can be activated by a variety of stimuli, including TNFα, IL-1β, and LPS. Activation of the complex involves the phosphorylation of two serine residues located in the "activation loop" within the kinase domain of IKKα and IKKβ. IKK complex is stimulated by upstream kinases that belong to MAP kinase kinase kinases (MAP3Ks), including MEKK1, MEKK2, MEKK3, and NIK, capable of phosphorylating these serines in vitro, and activating NF-κB.[23,24] Phosphorylation on specific serine residues of IκBs leads to ubiquitination of IκBs and subsequent degradation by the proteasome complex.

There is accumulating evidence suggesting the involvement of additional kinases that phosphorylate the p65 (RelA) subunit of NF-κB and regulate its transcriptional

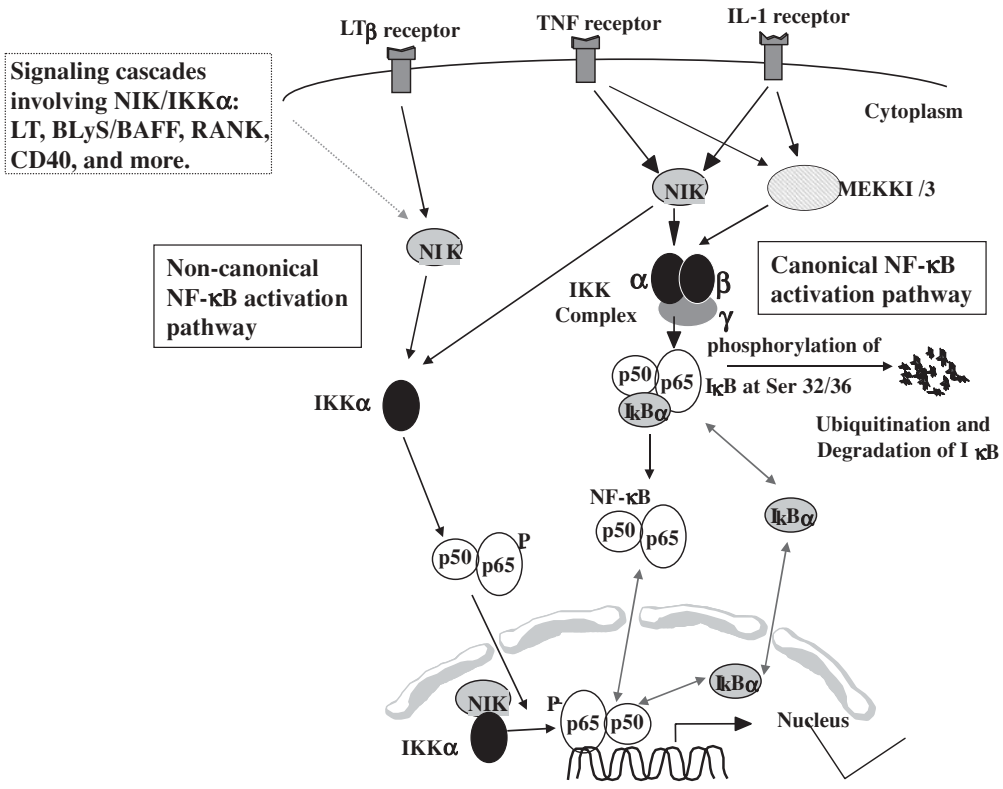

Fig. 2. NF-κB activation cascades. In addition to the canonical pathway involving IκB phosphorylation and ubiquitination followed by its proteolytic degradation in 26S proteasome within the cytoplasm, there appears to be another cascade not involving IκB phosphorylation. Lymphotoxin (*LT*) β-receptor signaling, CD40, RANK, and BLyS/BAFF stimulate the NIK–IKKα cascade that leads to p100/p52 processing and p65 phosphorylation at its C-terminal transactivation (Ser536). IKKα also phosphorylates histone H3 in the nucleus and derepresses the otherwise silent nucleosome, thus reactivating the dormant genes. The effect of p65 (Ser536) phosphorylation is considered to activate the transcriptional competence of NF-κB

Table 1. List of rheumatoid arthritis drugs that inhibit nuclear factor κB

Acetylsalicylic acid
Aurothioglucose
Aurothiomalate
Auranofin
Dexamethasone
Ibuprofen
Sodium salicylate
Sulfasalazine

competence.[25–27] We recently found that IKKα is responsible for the p65 phosphorylation at Ser536 upon the lymphotoxin β receptor signaling mediated by NIK, and induces NF-κB activation independently of the IκB phosphorylation and its degradation.[28–30] Interestingly, this NIK–IKKα cascade is also involved in BLyS/BAFF, RANK, and most likely CD40 signaling.[31,32] In contrast to the classical (or canonical) pathway involving IKKβ and the phosphorylation of IκB, this cascade ("non-canonical pathway") does not necessarily involve IKKβ and IκB phosphorylation but involves p100 (NFkB2) processing and p65 phospho-rylation (Fig. 2). Since BLyS/BAFF and CD40 signaling cascades induce B-cell activation and RANK signaling is involved in osteoclast differentiation, the NIK–IKKα cascade is considered to play important roles in disease progression of RA. The TNFα-dependent phosphorylation of Ser529 has also been demonstrated to increase the transcriptional activity of p65. For example, casein kinase II was implicated in the TNFα-dependent phosphorylation of p65 on Ser529.[33] It was shown that Ser529 and Ser536 of p65 were required for transcriptional activation of p65 by AKT and the IL-1β signaling.[30,34]

Inducible phosphorylation of p65 appears to function at many different levels, including conformational changes in the transcriptional activation domain and promoting association with coactivator proteins CBP/p300.[20] It is possible that the phosphorylation of p65 may lead to dissociation from corepressor proteins such as histone deacetylases and Groucho proteins (TLE/AES) and selective interaction with FUS/TLS coactivator protein.[35–37] Regarding the cross-talk with the camp–PKA cascade, although my group and others found that it downregulates the NF-κB-dependent gene expression presumably mediated by C/EBPβ,[38–40] it has also been reported that the catalytic subunit of PKA (PKAc), associated with the NF-κB/IκBα complex, upregulates the NF-κB-dependent gene expression by directly phosphorylating p65 on serine 276,[27] thus pending the physiological relevance.

Cytological characteristics of rheumatoid synoviocytes

Although it appears that NF-κB plays a major role in the pathophysiology of RA, there is no evidence to support the possibility that NF-κB or its signaling cascade is impaired in RA. To clarify the transformed-like nature of rheumatoid synoviocytes, my group have performed gene expression

profile analyses of synoviocytes.[41] When compared with control synoviocytes obtained from healthy individuals (upon injury) or osteoarthritis patients, we found that both platelet-derived growth factor (PDGF) receptor α and a chemokine, SDF-1, genes are activated in RA synoviocytes without any external stimulus. Gene knockout studies showed that PDGF receptor α is required for the development of limb joints. During the early developmental stages, PDGF and SDF-1 are known to act as chemotactic factors for fibroblasts[42] and macrophages,[43,44] respectively. It is possible that synovial fibroblasts (type B synoviocytes) and synovial-lining macrophage-like cells (type A) communicate with each other by producing SDF1A and PDGF, respectively, in order to form the primordial joint tissue during the early embryonic development (reviewed in Ref. 41). Thus, it is likely that rheumatoid synoviocytes may have reacquired the "revertant" phenotype of the primordial synoviocytes, like cancer cells, although the underlining mechanism is yet to be clarified.

As mentioned above, rheumatoid synovial tissues are usually under inflammatory stimuli as synovial fluid contains high concentrations of TNFα, IL-1, and oxidants (Ref. 46 and references therein). Thus, we extended the gene expression profile analysis with synoviocytes in the presence of physiological concentration (200 pg/ml) of TNFα and compared the genes induced by TNFα in RA and control synoviocytes. Although well-known genes under the control of NF-κB were similarly stimulated by TNFα, we found that the genes encoding Notch-1, Notch-4, and Jagged-2, a ligand for Notch proteins, were activated only in RA synoviocytes.[49] (Fig. 3). We also found that genes encoding MMP-11 and -17, and Wee1 and cyclin B1, were induced by TNFα only in rheumatoid synoviocytes. These findings indicate that one of the effects of phenotypic reversion of rheumatoid synoviocytes, as described above, could be attributable to the induction of Notch signaling and that the activation of Notch signaling, known to be involved in cell-fate determination, may directly or indirectly cause induction of genes responsible for cell proliferation (such as induction of Wee1 and cyclin B1 genes) and tissue invasion (such as induction of MMP-11 and -17). These findings support an idea that RA synoviocytes may have reacquired the "revertant" phenotype mimicking the primordial synoviocytes, by presumably involving Notch signaling, and exhibit the hyperproliferative and invasive nature of cells.

Activation of Notch signal in RA

As TNFα induced Notch-1 and its ligand Jagged-2 in RSF, we examined if the Notch signaling is elicited by the TNFα stimulation. Rheumatoid synovial fibroblasts (RSF) and normal synovial fibroblasts (NSF) were stimulated with TNFα and the intracellular localization of Notch intracellular domain (NICD) of Notch-1 was examined by immunostaining. We found the nuclear translocation of Notch-1 NICD, a hallmark of the Notch signaling,[48,49] only in TNFα-stimulated RSF[47] (Fig. 4). These results suggested that in response to TNFα stimulation RSF expressed both Notch-1 and Jagged-2 proteins, which then interacted with each other between adjacent cells and elicited the signaling. In RA tissues we found that hyperproliferative synovial tissues were clearly stained by Notch-1, Notch-4, and Jagged-2 antibodies, and that some of the RA synovial cells showed the nuclear staining of Notch-1 and Notch-4.

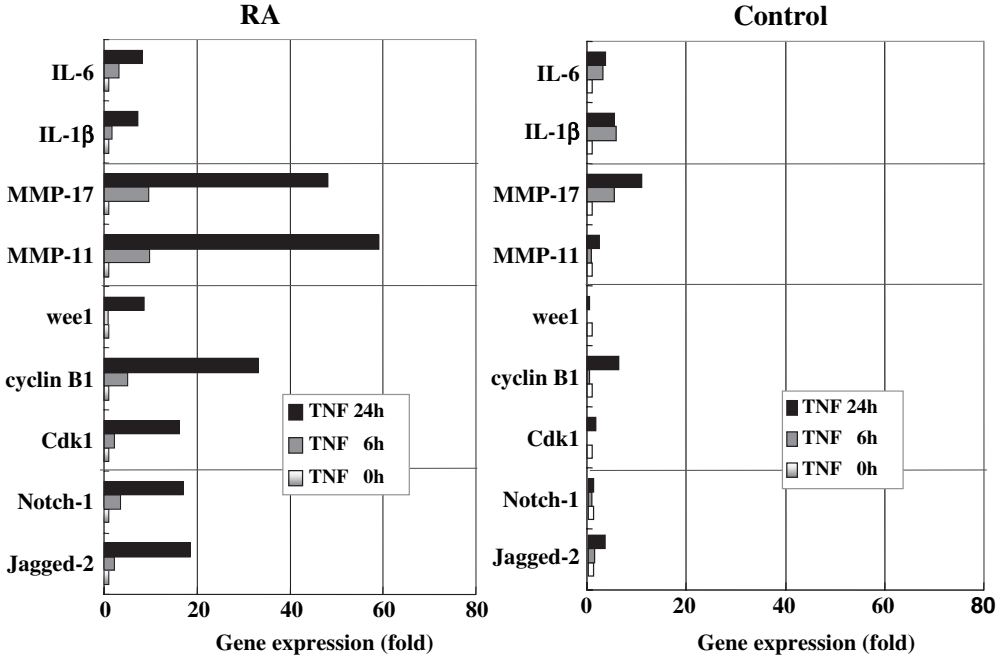

Fig. 3. Comparative gene expression profile analysis of rheumatoid and control synoviocytes upon stimulation with tumor necrosis factor α (*TNF*). Synoviocytes were cultured with or without TNF (200 pg/ml). The mRNA was purified from each cell culture harvested at 0, 6, and 24 h after TNF stimulation, cDNA probe was synthesized, then hybridized with a cDNA array membrane. The quantitation of gene expression level was performed and standardized based on the average levels of housekeeping genes. Based on the observation by Ando et al.[47] Reproduced with permission[47]

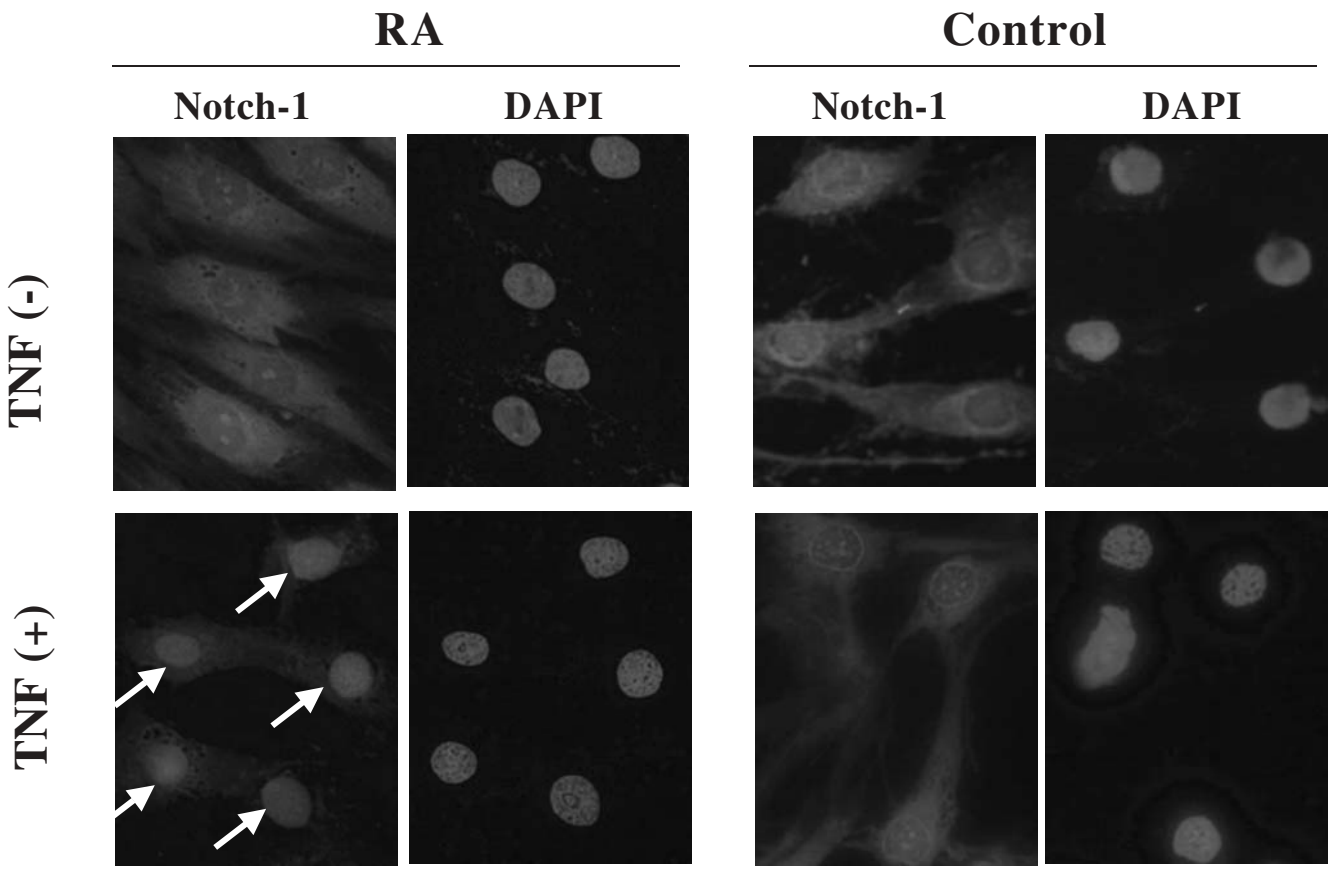

Fig. 4. Nuclear translocation of Notch-1 intracellular domain (NICD) in rheumatoid synoviocytes after tumor necrosis factor α (*TNF*) stimulation. Cells were immunostained with anti-Notch-1 C-terminus polyclonal antibody (C-20) before and after 12 h of TNF stimulation and examined by fluorescent microscopy. *Green*, Notch-1 intracellular domain (detected by fluorescein isothiocyanate-conjugated rabbit antigoat IgG as secondary antibody); *Blue*, nuclear staining with 4′,6′-diamidino-2-phenylindole hydrochloride (*DAPI*). Based on the observation by Ando et al.[47] Reproduced with permission[47]

We also detected expression of these proteins in the developing synovial and cartilage tissues of embryonic mice.[47] In more developed joints of newborn mice, expression of these proteins was restricted in the synovium, raising a possibility that the Notch signaling pathway might control the differentiation and development of joints.

Biological implications of Notch signal activation in RA

Notch signaling is involved in three different biological processes including (i) lateral specification, in which adjacent equipotent precursor cells coordinate each other's developmental fate, (ii) inductive signaling, in which one cell type determines the differentiation of another cell type, and (iii) cell-autonomous effects, in which a developing precursor (stem) cell regulates its own fate and maintains its status (reviewed in Refs. 50 and 51). Notch genes encode single-pass transmembrane receptors that transduce the extracellular signals responsible for cell fate determination during crucial steps of metazoan development.[52,53] The large transmembrane receptors encoded by Notch genes interact with membrane-bound ligands encoded by the Delta and Jagged (Serrate) genes at the extracellular surface of cells. The signal induced by this ligand binding leads to proteolysis of Notch, generation and nuclear translocation of NICD, and regulation of target gene expression (Fig. 5). Genes homologous to members of the Notch signaling pathway have been cloned from numerous vertebrate organisms and many have been shown to be essential for normal embryonic development. In humans, the importance of Notch signaling for growth and development is supported by the findings that T-lymphoblastic leukemia[54] and some inherited diseases involving affected organogenesis[55,56] can be ascribed to the mutations in Notch/Jagged (Delta) genes. The Notch signaling pathway is evolutionarily conserved, and mutations in its components disrupt cell fate specification and embryonic development in diverse organisms.[48,50]

Interestingly, a targeted mutation that removes a domain of the Jagged-2 protein required for the interaction with Notch-1 caused perinatal death associated with defects in craniofacial morphogenesis and syndactyly (digit fusions) of the fore- and hindlimbs, implicating that Jagged-2/Notch signaling is indispensable for the development of the joint.[57]

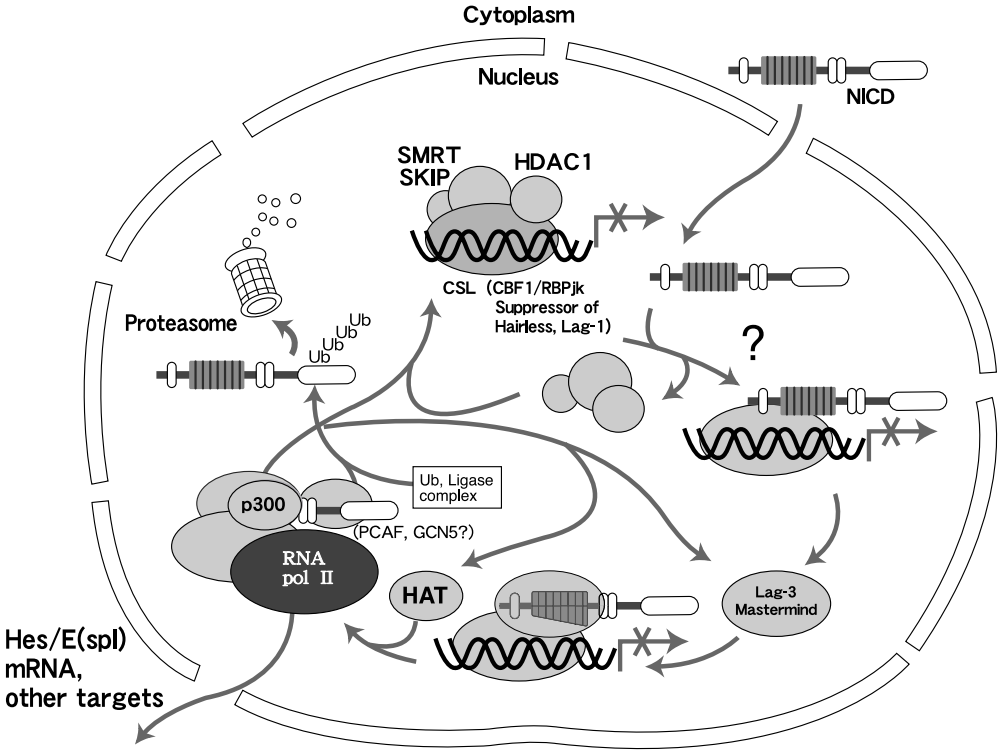

Fig. 5. Notch signaling and the transcriptional control by the Notch intracellular domain (*NICD*). Upon ligand binding, Notch protein is proteolytically cleaved by furin and presenilin to generate NICD, which is translocated to the nucleus. Once in the nucleus, NICD displaces the corepressor proteins such as *SKIP*, *SMRT*, and *HDAC-1* from the specific DNA-binding proteins *CSL* (CBP or RBP-Jκ in vertebrates, Su(H) in *Drosophila*, and Lag-1 in *Caenorhabditis elegans*; thus collectively called "CSL") and associates with the coactivator complex containing *Mastermind* (MAM) and *p300*;[62] MAM is considered to bridge the NICD/RBP–Jκ complex and p300.[63] The Notch intracellular domain is subjected to ubiquitination followed by proteasome-mediated degradation, thus terminating the transcriptional activation

A similar phenotype has been observed in mice lacking the IKKα subunit of IκB kinase complex.[58] In embryonic day 16 (E16) mutant embryos, forelimbs (but not hindlimbs) were visible but were considerably shorter than those of normal ($Ikk\alpha^{+/+}$ and $Ikk\alpha^{+/-}$) littermates and lacked separated digits. At an earlier stage, E14.5, the fore- and hindlimbs of mutant embryos were not much shorter than those of normal counterparts, but were devoid of distinct digits. Therefore, it appears that the TNF-mediated NF-κB activation through IKKα is involved in expression of Jagged-2 in the developing joints. Thus, activation of the Notch signaling found in rheumatoid synoviocytes not only confirms the phenotypic reversion of synoviocytes but also indicates its active role in pathophysiological processes of RA, which presumably involve NF-κB cascade.

Cross-talk between NF-κB activation cascade and Notch signaling

In mammals, all four known Notch family members can physically interact with recombinant signal binding protein Jκ (RBP-Jκ), a DNA-binding repressor protein, and inhibit the activity of RBP-Jκ.[59,60] Oswald et al.[61] reported that NICD overcame the RBP-Jκ-mediated repression and strongly activated NF-κB2. In the absence of Notch signaling, RBP-Jκ interacts with SKIP and SMRT that recruit transcriptional corepressor complex[62] (Fig. 5). However, upon Notch signaling NICD induces changes in the DNA-bound protein assembly containing RBP-Jκ in the nucleus, thus displacing the corepressor complex and converting it to a transcriptionally active complex. It has been shown that a non-DNA-binding transcriptional coactivator Mastermind (MAM) is essential for the Notch/RBP-Jκ complex to recruit p300 coactivator to DNA.[63] Thus, activation of Notch signaling observed in rheumatoid synoviocytes appears to stimulate the noncanonical NF-κB pathway (Fig. 5).

It is conceivable that this noncanonical NF-κB activation pathway may be responsible for the altered response to the inflammatory environment involving IKKα. It is known that IKKα is translocated, together with NF-κB, to the nuclear chromatin compartments where target genes are present, and phosphorylates Ser10 of the histone H3 component of nucleosome[64,65] (Fig. 5). Although the histone H3 with methylated lysine 9 of H3 renders the local nucleosome to be "repressive," the adjacent serine 10-phosphorylation of H3 histone reverses this effect and derepresses the transcriptional activity of the genes located in the "derepressed" nucleosome.[66] Thus, chronic and persistent NF-κB stimulation in synoviocytes of RA patients could also lead to the change in "histone code"[66] and eventually transform synoviocytes.

Conclusion

Rheumatoid arthritis is a complex process of chronic and progressive inflammation involving numerous transcription factors and signaling molecules. Based on the unexpected transcriptomic characteristics of rheumatoid synoviocytes, suggesting the phenotypic reversion, I have explored the mechanism by which chronic inflammatory stimuli could

endow normal synoviocytes with "transformed-like" phenotype and could ascribe activation of the Notch signaling to this altered cellular status. This may explain the progressive and self-perpetuating nature of the rheumatoid inflammation, at least in part. Based on these considerations, future therapeutic strategy of RA should be developed based on the action of Notch signaling on its pathophysiology, which includes the action of the noncanonical NF-κB activation pathway, its therapeutic intervention, and elucidation of the Notch target genes, particularly in synoviocytes.

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References

1. Fox DA. Etiology and pathogenesis of rheumatoid arthritis. In: Koopman WJ, editor. Arthritis and allied conditions – a textbook of rheumatology. Baltimore: Williams and Wilkins; 1997. p. 1085–101.
2. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.
3. Feldmann M. Pathogenesis of arthritis: recent research progress. Nat Immunol 2001;2:771–3.
4. Fassbender HG, Simmling-Annefeld M, Stofee E. Transformation of synovial rheumatoid arthritis. Verh Dtsch Ges Pathol 1980; 64:193–212.
5. Fassbender HG, Simmling-Annefeld M. The potential aggressiveness of synovial tissue in rheumatoid arthritis. J Pathol 1983;139, 399–406.
6. Firestein GS. Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Arthritis Rheum 1996;39:1781–90.
7. Kinne RW, Brauer R, Stuhlmutter B, Palombo-Kinne E, Burmester GR. Macrophages in rheumatoid arthritis. Arthritis Res 2000;3:189–202.
8. Okamoto T, Sakurada S, Yang JP, Merin JP. Regulation of NF-kappa B and disease control: identification of a novel serine kinase and thioredoxin as effectors for signal transduction pathway for NF-κB activation. Curr Top Cell Regul 1997;35:149–61.
9. Opferman JT, Korsmeyer SJ. Apoptosis in the development and maintenance of the immune system. Nat Immunol 2003;4:410–5.
10. Karin M, Lin A. NF-κB at the crossroads of life and death. Nat Immunol 2002;3:221–7.
11. Kajino S, Suganuma M, Teranishi F, Takahashi N, Tetsuka T, Ohara H, et al. Evidence that de novo protein synthesis is dispensable for anti-apoptotic effects of NF-κB. Oncogene 2000;19:2233–9.
12. Yang JP, Hori M, Takahashi N, Kawabe T, Kato H, Okamoto T. NF-κB subunit p65 binds to 53BP2 and inhibits cell death induced by 53BP2. Oncogene 1999;18:5177–86.
13. Takahashi N, Kobayashi S, Jiang X, Kitagori K, Imai K, Hibi Y, et al. Expression of 53BP2 and ASPP2 proteins from TP53BP2 gene by alternative splicing. Biochem Biophys Res Commun 2004; 315:434–8.
14. Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M. Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet 1989;2: 244–7.
15. Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 antagonist. Arthritis Rheum 1998;41: 2196–204.
16. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. J Clin Invest 2001;107:135–42.
17. Yang JP, Merin JP, Nakano T, Kato T, Kitade Y, Okamoto T. Inhibition of the DNA-binding activity of NF-kappa B by gold compounds in vitro. FEBS Lett 1995;361:89–96.
18. Yoshida S, Kato T, Sakurada S, Kurono C, Yang JP, Matsui N, et al. Inhibition of IL-6 and IL-8 induction from cultured rheumatoid synovial fibroblasts by treatment with aurothioglucose. Int Immunol 1999;11:151–8.
19. Baldwin AS Jr. Series introduction: the transcription factor NF-κB and human disease. J Clin Invest 2001;107:3–6.
20. Schmitz ML, Bacher S, Kracht M. IκB-independent control of NF-κB activity by modulatory phosphorylations. Trends Biochem Sci 2001;26:186–90.
21. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol 2000;18:621–63.
22. Chen ZJ, Parent L, Maniatis T. Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity. Cell 1996;84:853–62.
23. Yamamoto Y, Verma UN, Prajapati S, Kwak YT, Gaynor RB. Histone H3 phosphorylation by IKK-a is critical for cytokine-induced gene expression. Nature 2003;423:655–9.
24. Ozaki M, Deshpande SS, Angkeow P, Bellan J, Lowenstein CJ, Dinauer MC, et al. Inhibition of the Rac1 GTPase protects against nonlethal ischemia/reperfusion-induced necrosis and apoptosis in vivo. FASEB J 2000;14:418–29.
25. Hayashi T, Sekine T, Okamoto T. Identification of a new serine kinase that activates NF kappa B by direct phosphorylation. J Biol Chem 1993;268:26790–5.
26. Naumann M, Scheidereit C. Activation of NF-κB in vivo is regulated by multiple phosphorylations. EMBO J 1994;13:4597–607.
27. Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-κB p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell 1998;1: 661–71.
28. Jiang X, Takahashi N, Matsui N, Tetsuka T, Okamoto T. The NF-κB activation in the lymphotoxin beta receptor signaling depends on the phosphorylation of p65 at serine 536. J Biol Chem 2003;278:919–26.
29. Jiang X, Takahashi N, Ando K, Otsuka T, Tetsuka T, Okamoto T. NF-κB p65 transactivation domain is involved in the NF-κB-inducing kinase (NIK) pathway. Biochem Biophys Res Commun 2003;301:583–90.
30. Sakurai H, Chiba H, Miyoshi H, Sugita T, Toriumi W. IκB kinases phosphorylate NF-κB p65 subunit on serine 536 in the transactivation domain. J Biol Chem 1999;274:30353–6.
31. Cao Y, Bonizzi G, Seagroves TN, Greten FR, Johnson R, Schmidt EV, et al. IKKα provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. Cell 2001;107:763–75.
32. Ghosh S, Karin M. Missing pieces in the NF-κB puzzle. Cell 2002; 109:S81–96.
33. Wang D, Baldwin AS Jr. Activation of nuclear factor-kappa B-dependent transcription by tumor necrosis factor-alpha is mediated through phosphorylation of RelA/p65 on serine 529. J Biol Chem 1998;273:29411–6.
34. Madrid LV, Wang CY, Guttridge DC, Schottelius AJ, Baldwin AS Jr, Mayo MW. Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-κB. Mol Cell Biol 2000;20:1626–38.
35. Uranishi H, Tetsuka T, Yamashita M, Asamitsu K, Shimizu M, Itoh M, et al. Involvement of the pro-oncoprotein TLS (translocated in liposarcoma) in nuclear factor-kappa B p65-mediated transcription as a coactivator. J Biol Chem 2001;276:13395–401.
36. Tetsuka T, Uranishi H, Imai H, Ono T, Sonta S, Takahashi N, et al. Inhibition of nuclear factor-kappa B-mediated transcription by association with the amino-terminal enhancer of split, a Groucho-related protein lacking WD40 repeats. J Biol Chem 2000;275:4383–90.
37. Ashburner BP, Westerheide SD, Baldwin AS Jr. The p65 (RelA) subunit of NF-κB interacts with the histone deacetylase (HDAC)

corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol 2001;21:7065–77.
38. Neumann M, Grieshammer T, Chuvpilo S, Kneitz B, Lohoff M, Schimpl A, et al. RelA/p65 is a molecular target for the immunosuppressive action of protein kinase A. EMBO J 1995;14:1991–2004.
39. Ollivier V, Parry GC, Cobb RR, de Prost D, Mackman N. Elevated cyclic AMP inhibits NF-κB-mediated transcription in human monocytic cells and endothelial cells. J Biol Chem 1996;271:20828–35.
40. Takahashi N, Tetsuka T, Uranishi H, Okamoto T. Inhibition of NF-κB transcriptional activity by protein kinase A. Eur J Biochem 2002;269:1–7.
41. Watanabe N, Ando K, Yoshida S, Inuzuka S, Kobayashi M, Matsui N, et al. Gene expression profile analysis of rheumatoid synovial fibroblast cultures revealing the overexpression of genes responsible for tumor-like growth of rheumatoid synovium. Biochem Biophys Res Commun 2002;294:1121–9.
42. Postlethwaite AE, Seyer JM. Fibroblast chemotaxis induction by human recombinant interleukin-4: identification by synthetic peptide analysis of two chemotactic domains residing in amino acid sequences 70–88 and 89–122. J Clin Invest 1991;87:2147–52.
43. Burger JA, Zvaifler NJ, Tsukada N, Firestein GD, Kipps TJ. Fibroblast-like synoviocytes support B-cell pseudoemperipolesis via a stromal cell-derived factor-1-and CD106 (VCAM1)-dependent mechanism. J Clin Invest 2001;107:305–15.
44. Muller A, Homey B, Soto H, Ge D, Buchanan ME, McClanahan T, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–6.
45. Soriano P. The PDGFα receptor is required for neural crest cell development and for normal patterning of somites. Development 1997;124:2691–700.
46. Yoshida S, Katoh T, Tetsuka T, Uno K, Matsui N, Okamoto T. Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNFα-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. J Immunol 1999;163:351–8.
47. Ando K, Kanazawa S, Tetsuka T, Ohta S, Jiang X, Tada T, et al. Induction of Notch signaling by tumor necrosis factor in rheumatoid synovial fibroblasts. Oncogene 2003;22:7796–803.
48. Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. Nature 1998;393:382–6.
49. Struhl G, Greenwald I. Presenilin is required for activity and nuclear access of Notch in Drosophila. Nature 1999;398:522–5.
50. Greenwald I. Lin-12/Notch signaling: lessons from worms and flies. Genes Dev 1998;12:1751–62.
51. Artavanis-Tsakonas S, Matsuno K, Fortini ME. Notch signaling. Science 1995;268:225–32.
52. Fortini ME, Artavanis-Tsakonas S. The suppressor of hairless protein participates in notch receptor signaling. Cell 1994;79:273–82.
53. Matsuno K, Diederich RJ, Go MJ, Blaumueller CM, Artavanis-Tsakonas S. Deltex acts as a positive regulator of Notch signaling through interactions with the Notch ankyrin repeats. Development 1995;121:2633–44.
54. Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 1991;66:649–61.
55. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996;383:707–10.
56. Li L, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat Genet 1997;16:243–51.
57. Jiang R, Lan Y, Chapman HD, Shawber C, Norton CR, Serreze DV, et al. Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. Genes Dev 1998;12:1046–57.
58. Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, et al. Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase. Science 1999;284:316–20.
59. Kuroda K, Tani S, Tamura K, Minoguchi S, Kurooka H, Honjo T. Delta-induced Notch signaling mediated by RBP-J inhibits MyoD expression and myogenesis. J Biol Chem 1999;274:7238–44.
60. Aster JC, Robertson ES, Hasserjian RP, Turner JR, Kieff E, Sklar J. Oncogenic forms of NOTCH1 lacking either the primary binding site for RBP-Jkappa or nuclear localization sequences retain the ability to associate with RBP-Jkappa and activate transcription. J Biol Chem 1997;272:11336–43.
61. Oswald F, Liptay S, Adler G, Schmid RM. NF-kappaB2 is a putative target gene of activated Notch-1 via RBP-Jkappa. Mol Cell Biol 1998;18:2077–88.
62. Zhou S, Hayward SD. Nuclear localization of CBF-1 is regulated by interactions with the SMRT corepressor complex. Mol Cell Biol 2001;21:6222–32.
63. Fryer CJ, Lamar E, Turbachova I, Kintner C, Jones KA. Mastermind mediates chromatin-specific transcription and turnover of the Notch enhancer complex. Genes Dev 2002;16:1397–411.
64. Anest V, Hanson JL, Cogswell PC, Steinbrecher KA, Strahl BD, Baldwin AS. A nucleosomal function for IκB kinase-α in NF-κB-dependent gene expression. Nature 2003;423:659–63.
65. Yamamoto Y, Verma UN, Prajapati S, Kwak YT, Gaynor RB. Histone H3 phosphorylation by IKK-a is critical for cytokine-induced gene expression. Nature 2003;423:655–9.
66. Fischle W, Wang Y, Allis CD. Binary switches and modification cassettes in histone biology and beyond. Nature 2003;425:475–9.