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REVIEW ARTICLE

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Implications of transcriptional coactivator CREB binding protein complexes in rheumatoid arthritis

Abstract Transcriptional coactivators have crucial roles in eukaryotic transcription. It has been suggested that one of the coactivators, cAMP response element binding protein (CREB) binding protein (CBP), regulates gene expression with a number of transcription factors via two mechanisms. One is the recruitment of general transcriptional machinery to the promoters. The other is its intrinsic and associated histone acetyltransferase (HAT) activity, which increases the accessibility of the activator to DNA, and the acetylation of nonhistone proteins. Rheumatoid arthritis (RA) is characterized by the inflammation and proliferation of synovium, leading to the destruction of articular cartilage and bone. To understand the pathogenesis of RA, we focused the transcription mechanism through CBP in synoviocytes and chondrocytes. We identified Notch-1 in synoviocytes and $p34^{SEI-1}$ in chondrocytes as CBP binding proteins by yeast two-hybrid screening. It was also suggested that the acetylation of p53 could repress transactivation in RA synoviocytes. These associations may regulate proliferation and apoptosis. This study suggests that regulation of the coactivator could become a novel strategy for RA therapy.

Key Words CREB binding protein (CBP) · Coactivator · Histone acetyltransferase (HAT) · Rheumatoid arthritis (RA) · Synoviocyte · Transcription

Introduction

A cell responds to physiological and environmental cues through the regulation of gene expression. In eukaryotic cells, genes form nucleosome complexes with core histones and other chromosome proteins to generate chromatin. The nucleosome includes two copies of four core histones, H2A, H2B, H3, and H4, wrapped by 146 bp of DNA.[1] Since this structure represses transcription, some transactivation mechanism is necessary so that the transcriptional machinery gains access to specific loci on DNA. One mechanism is mediated by chromatin remodeling complexes, while another is histone acetylation. The histone acetyltransferase (HAT) enzymes transfer an acetyl group to the lysine residues on N-terminal histone tails. These modifications increase the accessibility of transcription factors to the DNA by changing the charge of the histone tails and destabilizing the chromatin structure.[2,3] Recently, the acetylation of histones was shown to function as a marker for the regulation of transcriptional activation or silencing. Some modifications of histones (phosphorylation, methylation, ubiquitylation, and sumolylation) as well as acetylation are important for transcriptional regulation. The enzymes modifying the histone tails are highly specific for particular amino acid positions. The combination of these modifications on the same or different histone tails, called the "histone code," is predicted to induce interactions with chromatin-associated proteins.[4,5]

Eukaryotic gene expression is also mediated by the recruitment of basal transcriptional machinery, including general transcription factors and RNA polymerase (Pol) II.[6,7] It has been reported that several nuclear factors, called "coactivators," play important roles in the recruitment of Pol II and the enzymatic conversion of the chromatin structure in transactivation. One of the most characterized coactivators, cAMP response element binding protein (CREB) binding protein (CBP), also activates transcription through interaction with the general transcription machinery and its enzymatic activity.[8,9]

In 1993, CBP was originally identified as a binding protein with the transcription factor CREB-phosphorylated serine residue at position 133.[10] CREB stimulates target gene expression by the recruitment of transcriptional machinery through two mechanisms. CREB has bipartite transactivation domains consisting of a constitutive and inducible activator termed Q2, and a kinase inducible domain

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(KID).[11,12] These domains synergistically function in response to a cAMP signal. The glutamine-rich Q2 domain engages the transcriptional apparatus via a constitutive interaction with $hTAF_{II}130$.[13,14] In contrast, KID modulates CREB activity via a phosphorylation-dependent association with coactivator CBP.[15,11] CBP interacting with phosphorylated CREB also activates the transcription via two mechanisms. One is dependent on its intrinsic and associated HAT activity,[16,17] and the other is the recruitment of Pol II mediated by RNA helicase A (RHA).[18,19] CBP allows the acetylation of all core histones, and activates transcription (Fig. 1). Recent reports have shown that HATs could also acetylate molecules such as p53,[20] GATA-1,[21] and TFIIE[22] as well as histones, and function as factor acetyltransferases (FAT). CBP is reported to have FAT activity and acetylates many nuclear factors.

CBP is a large protein of 265 kD and consists of several functional domains, including three cysteine/histidine-rich regions (CH1, CH2, and CH3), one bromo domain, a HAT domain, and a C-terminal glutamine-rich domain (Fig. 2).[10,23] This protein associates not only with CREB, but also with a number of transcriptional activators such as nuclear receptors,[24] NF-κB,[25] etc., via such domains. These factors recruit CBP to specific promoters in response to each nuclear signal. Some pathways interfere with one another by competing for the recruitment of this protein.[26,27] These findings suggest that CBP functions as an integrator to respond to various stimuli and activate the transcription.[24]

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by synovial hyperplasia and infiltration of T cells, leading to destruction of the cartilage in multiple joints.[28] We have reported that CBP is implicated in RA.[29–31] In this review, we describe the association between the transcriptional coactivator and arthritis.

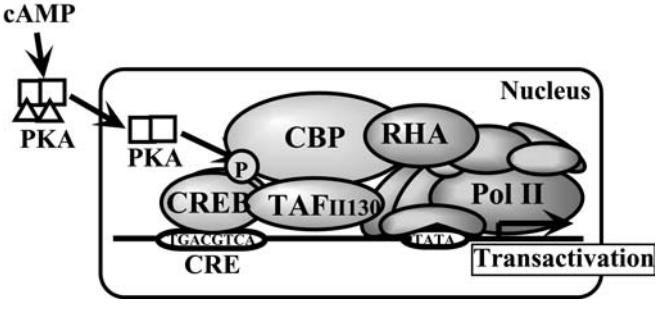

Fig. 1. Schematic representation of CREB-dependent transcription. CREB phosphorylated by a PKA signal interacts with coactivator CBP. CBP recruits Pol II complexes through RHA and activates the transcription

TNF-α induced the acetylation of p53 in RA synoviocytes

Rheumatoid fibroblast-like synoviocytes are activated and proliferate, and escape the normal growth regulatory mechanism.[32] Recent studies have shown that acetylation could influence the proliferation, differentiation, and apoptosis of cells.[33–35] To examine the state of nuclear acetylation in RA synoviocytes, we used an antibody against acetylated lysine.[36] In the immunohistochemistry of tissue from patients, strong nuclear acetylation was detected in RA synovium, whereas only weak staining was detected in osteoarthritis (OA) synovium. An enhancement of acetylation was also found in cultured rheumatoid synoviocytes compared with those derived from OA. To analyze the acetylated proteins in the nuclei of RA synoviocytes, Western blotting with antiacetylated lysine antibody was performed. This analysis showed that approximately 53 and 62 kD proteins were highly acetylated in the nuclei from cultured RA synoviocytes.

Tumor necrosis factor (TNF)-α is known to be a crucial cytokine in the activation of synoviocytes.[37] We assessed the effects of TNF-α on hypernuclear acetylation in RA synoviocytes. As a result of treatment with TNF-α, the acetylation of 53 and 62 kD proteins increased. The same assay with RA patients showed a slight enhancement of acetylation.

Tumor suppressor protein p53 is acetylated at the multiple lysine residues in the C-terminal region,[38] and relates to apoptosis and cell proliferation through transcriptional regulation.[39] It was also reported that some functional mutations in the p53 protein are observed in RA patients.[40–42] These reports prompted us to check whether the acetylated 53 kD protein observed in RA synoviocytes is p53. The immunoprecipitated 53 kD protein with antiacetylated lysine antibodies from nuclear extract derived from RA synoviocytes was also detected by a specific antibody against p53. These results suggested that p53 was highly acetylated in RA synoviocytes. Another acetylated 62 kD protein remains to be checked.

As well as mutations of p53, it has been reported that the inhibition of endogenous p53 expression leads to increased invasiveness, and p53-deficient mice with collagen-induced arthritis show a more severe phenotype than wild type.[43] We

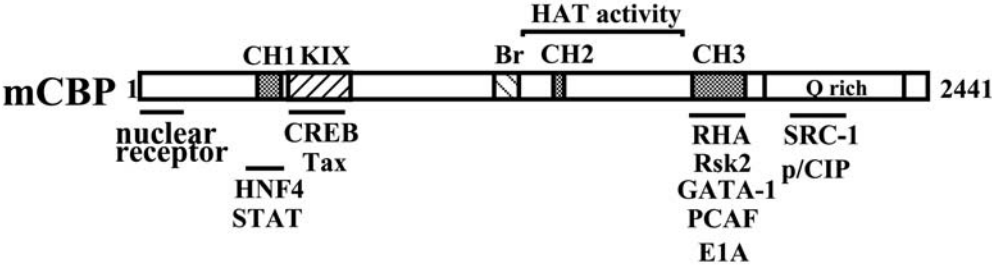

Fig. 2. Schematic representation of the primary structure of CBP. Known interacting factors and their binding regions are indicated

focused on the acetylation of p53, which has a transactivation domain in the N-terminus, and a DNA binding domain in its middle region. In the C-terminal region, it contains a tetramerization domain and a basic regulatory domain. It has been shown that CBP acetylates lysine residues at 372, 373, and 381 positions and PCAF at 382 within the C-terminal of p53, and these acetylations could increase the accessibility to DNA by the conformational change.[38,44] p53 functions as a DNA sequence-specific transcription factor and regulates the transcription of the cell cycle, DNA repair, and apoptosis-related genes.[39]

We then performed a gel-shift assay using TNF-α-treated nuclear extract to examine whether acetylations of p53 had an effect on its DNA binding activity. As a result, p53 bound to the specific DNA in a TNF-α dose-dependent manner. This finding suggested that TNF-α augmented the binding of p53 to the promoter and mediated the acetylation.

We explored the relation between the acetylation of p53 by TNF-α signals and transactivation. A luciferase assay using the reporter with a p53 binding element was performed on RA synoviocytes. TNF-α decreased the p53-dependent transcriptional activity, whereas IL-1-β, bFGF, and IL-6, which are known as cytokines no rich activate synoviocytes, had no effect on transactivation via p53. It has been reported that the acetylation of p53 relates to the recruitment of coactivators.[45] The effects of CBP, a coactivator with HAT activity, were examined. CBP rescued the attenuation on transactivation by TNF-α. Interestingly, mutant CBP lacking HAT activity also blocked the TNF-α effect. Furthermore, this transcriptional repression-mediated p53 acetylation was not observed in any cells except RA synoviocytes.

We have reported that TNF-α plays an important role as a mitogenic cytokine for rheumatoid synoviocytes. Since this finding indicated that CBP rescued the transcriptional suppression mediated by acetylated p53 in RA synoviocytes, we hypothesized that a sufficient amount of CBP may induce apoptosis by TNF-α in RA synoviocytes. As a result, presence of typical concentrated DNA was detected in CBP-expressing cells treated with TNF-α. This indicates that the overexpression of CBP induced apoptosis-like features in RA synoviocytes. Together with these studies, it is suggested that the inhibition of p53/CBP-dependent transactivation by TNF-α signals could suppress apoptosis, which may lead to a proliferation of synoviocytes.

Recruitment of CBP and transactivation in the joint

As described above, CBP is a coactivator associating with a number of transcription factors. CBP is expressed ubiquitously, and interacts with tissue or time-specific factors such as MyoD,[46] GATA-1,[47] and PPAR-γ[48] to control the differentiation of cells. These reports made us hypothesize that the disfunction of CBP-mediated transcription might cause disease. This proposal prompted us to search for CBP binding proteins in RA synovium. We performed yeast two-hybrid screening using the CH3 region of CBP, which interacts with RHA,[18] Rsk2,[27] GATA-1,[47] NeuroD,[49] etc. (see Fig. 2).

Notch-1 pathway activated in RA synoviocytes

The screenings with a library derived from RA synoviocytes found Notch-1 to be a CBP binding protein. Notch was first discovered to be an important factor in the differentiation and determination of cell fate in *Drosophila melanogaster*.[50] The Notch signaling pathway is highly conserved from nematode to human.[51–54] Notch is a single-pass transmembrane receptor that forms a heterodimer of 300 kD. An extracellular 180-kD subunit (p180) has 36 EGF-like repeats, Lin/Notch repeats (LNR). An intracellular 120-kD subunit consists of a transmembrane domain, ankyrin repeats, a RAM domain, and a PEST sequence,[50] and is cleaved at the TM domain by γ-secretase for ligand-binding dependence. The intracellular region (Notch intracellular domain, NICD) transfers into the nucleus[55–57] and activates the transcription of target genes such as *Enhancer of split* [E(spl)] and Hes with transcription factor CBF1, suppressor of hairless, Lag-1 (CSL).[58]

We analyzed the functions of the Notch-1 found as a CBP binding protein in RA synoviocytes. First, the expression of Notch-1 protein in RA patients was confirmed. Notch-1 was detected by immunohistochemistry in the nucleus of RA synovium, whereas it was localized in the cytoplasm of OA sinovium. Western blotting gave the same results.[30] These results suggested that the Notch-1 pathway was activated in RA synoviocytes.

Since Notch-1 is known to activate transcription,[58] we studied the function of NICD on transactivation in synoviocytes. We studied the expression mechanisms of the NF-κB2 gene that is reported to be a target and to relate to the suppression of apoptosis. NF-κB2 promoter activity is regulated by the two κB elements, and one of these elements overlaps with the consensus recombination signal binding protein Jκ (RBP-Jκ) binding site.[59,60] Without a NF-κB signal, RBP-Jκ, a mammalian homolog of CSL, represses the transactivation via the formation of a co-repressor complex with SMRT/histone deacetylase (HDAC). When Notch-1 is activated and internalized to the nucleus, NICD interacts with RBP-Jκ and dissociates the co-repressor complex from the κB element. This allows the NF-κB to bind with the κB elements and activate the transcription of target genes.[58,60] Since Notch-1 associates with HAT like PCAF, GCN5,[61] and CBP,[62] it could relate positively with transactivation as well as the release of the co-repressor complex. On the other hand, Notch-1 could convert RBP-Jκ into a transcriptional activator.

First, we examined the expression of NF-κB2 in synoviocytes. NF-κB2 expression was significantly elevated in RA synoviocytes compared with those from OA, and the cells activating the NF-κB2 expression corresponded to NICD expressed cells. A gel-shift assay revealed that NF-κB2 bound to κB elements in RA synoviocytes, while RBP-Jκ bound to the binding element in those derived from OA.

An immunoprecipitation assay indicated that Notch-1 formed a complex with RBP-Jκ in RA synoviocytes.[29] These results suggested that NICD activated the transcription of the NF-κB2 gene in RA synoviocytes, and the elevation of NF-κB2 expression may repress apoptosis in proliferating RA synoviocytes.

To analyze the Notch-1 activation mechanisms, we then assessed the effects of TNF-α on the translocation of NICD into the nucleus. NICD was detected in the nucleus of normal synoviocytes treated with TNF-α by Western blotting and immunofluorescence staining. This finding suggested that TNF-α, a cytokine that is crucial for synoviocyte activation, induced Notch-1 nuclear translocation.

To examine the effects of Notch-1 on RA synoviocyte proliferation, we designed antisense oligonucleotides. Antisense Notch-1 oligonucleotides appeared to inhibit the proliferation of synoviocytes derived from RA patients, whereas they had no effect on the proliferation of those from OA. TNF-α enhanced the proliferation of synoviocytes and antisense oligonucleotides blocked it in a dose-dependent manner.

Presenilin is a core component of γ-secretase that cleaves Notch-1 and releases NICD.[56,57] To confirm whether presenilin cleaves Notch-1 in RA synoviocytes, we treated the cells with MW167, which is an inhibitor of γ-secretase.[63] This resulted in an inhibition of translocation of NICD in RA synoviocytes. MW167 also blocked the proliferation of synoviocytes treated with TNF-α. Interestingly, the IL-1β-dependent proliferation of synoviocytes, which is also known to be strongly mitogenic for synoviocytes, was not affected by MW167. These results suggested that γ-secretase inhibitor selectively inhibited synoviocytes proliferation.[30] These findings prompted us to think of the possibility that γ-secretase inhibitor could block the proliferation of the cells in synovium from RA patients, and that γ-secretase may be useful for the treatment of rheumatoid arthritis.

Alzheimer's disease (AD) is known as a disease related to γ-secretase disorders. This disease is pathologically characterized by intraneuronal tangles and cerebral plaques containing the amyloid β peptide (Aβ) derived from the Aβ precursor protein (APP).[64] These are thought to cause the progressive loss of memory and general cognitive decline. APP, an integral membrane protein, is cleaved by β-secretase to a soluble version (β-APP_S) and a 99 residue-C terminal fragment (C99) that remains membrane-bound. The γ-secretase works in the middle of the transmembrane of C99 to generate 4kD Aβ. Most Aβ is a 40-amino acid peptide ($Aβ_{40}$), but a small population of Aβ is a 42-kD peptide ($Aβ_{42}$).[65,66]

The γ-secretase is a high-molecular-weight complex containing presenilin and a set of cofactors. In mammalian cells there are two presenilin genes (PS1 and PS2) that share 65 % identity, and they are thought to be a catalytic subunit of the complex.[67] Presenilin was identified as a causative gene of familial Alzheimer's disease (FAD). More than 80 FAD mutations in PS1 and PS2 have been reported, and all these mutations result in specific increases in $Aβ_{42}$. The longer and more hydrophobic $Aβ_{42}$ is likely to form a fibril structure, and it is believed that it relates to the pathogenesis of AD.[68,69]

Analyses with PS1- and PS2-deficient mice showed that presenilin is a catalytic subunit of γ-secretase for Norch-1.[70,71] It has been reported that nonsteroidal anti-inflammatory drugs (NSAIDs) help to prevent AD.[72] As described above, a relationship between AD and RA has been suggested. Furthermore, our findings clarified the existence of presenilin as a common factor between AD and RA. PS-deficient mice are usually embryonic lethal,[73] and presenilin is able to cleave both APP and Notch.[74] Although we should consider these problems, the γ-secretase inhibitor is thought to be a candidate drug for AD. These prompted us to hypothesize that drugs for AD are useful in the treatment of RA patients, since NSAIDs prevent the occurrence of AD. A γ-secretase inhibitor was reported which shows a significant difference in its capacity to inhibit APP and Notch. It is expected that drugs to treat RA which also target γ-secretase will be developed.

$p34^{SEI-1}$ associates with CBP in chondrocytes

In rheumatoid arthritis, patients experience erosion of articular cartilage that involves the degradation of type II collagen and proteoglycans. Chondrocytes synthesize the collagens and aggrecan of cartilage, and the stimulated condrocytes also secrete enzymes that degrade the matrix.[28] To identify the factors controlling the proliferation, differentiation, and activation of chondrocytes, we performed a yeast two-hybrid screening test with a library derived from chondrocytes stimulated with TGF-β and IL-1.[32] This resulted in the identification of $p34^{SEI-1}$ containing NLS, LXXLL motifs, and a MDM-like region. $p34^{SEI-1}$ was originally identified as a $p16^{INK4a}$ interacting protein. $p34^{SEI-1}$ interacts with CDK4.[75]

We confirmed the formation of CBP and $p34^{SEI-1}$. Binding assays indicated that the CH3 region interacted with a broad region of $p34^{SEI-1}$ (1–174 amino acids), and formed the complex in vivo. This complex co-localized with promielocytic leukemia (PML) which could be included in transcription[76] and exhibited a speckled pattern in the nucleus. Next, the effects of $p34^{SEI-1}$ on the functions of CBP were examined using reporter assays. $p34^{SEI-1}$ suppressed CRE-dependent transcription, and CBP rescued this suppression via $p34^{SEI-1}$. These results suggested that $p34^{SEI-1}$ might compete with transcriptional activators for association with CBP. It has been reported that $p34^{SEI-1}$ promotes cell cycle in some mechanisms.[75,77] One mechanism is the suppression of $p16^{INK4a}$. $p16^{INK4a}$ interacts with CDK4 and prevents CDK4 from binding with cyclin D. $p34^{SEI-1}$ associates with CDK4 and renders the cyclin D–CDK4 complex resistant to the inhibitory effect of $p16^{INK4a}$.[75] Another mechanism is E2F-dependent. $p34^{SEI-1}$ interacts with the DP-1 involved in the E2F complex and activates transcription from E2F-responsive promoters as a cofactor between the G1 and S phases.[77] CBP is known to activate E2F-dependent transcription as a coactivator.[78] In our study, $p34^{SEI-1}$ repressed CREB-dependent transcription. CREB

has also been reported to stimulate cell differentiation,[79] and our results are consistent with this report. These findings suggest that p34^{SEI-1} could regulate cell proliferation and differentiation.

Conclusions

We have shown the possibility that transcriptional coactivator CBP could regulate cell proliferation and apoptosis through its HAT activity and recruitment of the general transcriptional machinery in rheumatoid arthritis. However, particular mechanisms in each pathway via CBP remain to be clarified. Confirmation of the recruitment roles of CBP may clarify the mechanisms of hyperplasia and differentiation of cells in the joints, and lead to an investigation of the onset mechanism and therapy of rheumatoid arthritis. These studies suggest that transcriptional coactivator CBP may become a novel treatment for some disease.

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