Background: TCF/LEF transcription factors are downstream effectors of Wnt/β-catenin signaling. This was due to potentiating NF-κB signaling by a protein-protein interaction between TCF4 and β-catenin p65 activating established NF-κB target genes such as MMPs and IL-6. TCF1 competed with TCF4 for binding to NF-κB p65. IκB-α was able to counteract the effect of TCF4 on NF-κB target gene expression. Finally, we showed that TCF4 mRNA expression was elevated in OA cartilage compared with healthy cartilage and induced chondrocyte apoptosis at least partly through activating caspase 3/7. Our findings suggest that increased TCF4 expression may contribute to cartilage degeneration in OA by augmenting NF-κB signaling.

T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors are downstream effectors of Wnt/β-catenin signaling, which has been implicated in the development and progression of osteoarthritis (OA). This study aimed to investigate the role of TCF/LEF transcription factors in human articular chondrocytes. Primary human osteoarthritic cartilage predominantly expressed TCF4 and to a lesser extent, LEF1 and TCF3 mRNA. Overexpression of TCF4, but not of TCF3 or LEF1, induced MMP-1, -3, and -13 expression and generic MMP activity in human chondrocytes. This was due to potentiating NF-κB signaling by a protein-protein interaction between TCF4 and NF-κB p65 activating established NF-κB target genes such as MMPs and IL-6. TCF1 competed with TCF4 for binding to NF-κB p65. IκB-α was able to counteract the effect of TCF4 on NF-κB target gene expression. Finally, we showed that TCF4 mRNA expression was elevated in OA cartilage compared with healthy cartilage and induced chondrocyte apoptosis at least partly through activating caspase 3/7. Our findings suggest that increased TCF4 expression may contribute to cartilage degeneration in OA by augmenting NF-κB signaling.

Canonical Wnt signaling is a conserved signaling pathway implicated in many aspects of development and disease (1, 2). In the absence of Wnt, a destruction complex mediates the phosphorylation of β-catenin by glycogen synthase kinase-3β, which induces degradation of cytosolic β-catenin through the proteasome. Binding of Wnt to its receptors results in disruption of the destruction complex and accumulation of cytoplasmic β-catenin. Upon nuclear translocation, β-catenin will function as a co-factor of TCF/LEF transcription factors to switch on Wnt target gene transcription (3). Mammals have four TCF/LEF family members: TCF1, TCF3, TCF4, and LEF1 (4). Each member is produced as a group of isoforms through alternative splicing and promoter usage. The N-terminal β-catenin-binding domain of all four TCF/LEF members is highly conserved and responsible for the binding of β-catenin. The context-dependent regulatory domain and C-terminal tails are varied among all four members, resulting in different binding properties.

Cumulating studies mainly based on experimental animal models for OA have suggested an important pro-catabolic role for Wnt/β-catenin signaling in the pathogenesis of OA by stimulating, among others, hypertrophic differentiation of chondrocytes and the expression of matrix degrading MMPs in articular cartilage (5, 6). Indeed, in animal chondrocytes it has been demonstrated that MMPs are direct β-catenin/Tcf target genes and that IL-1β-induced MMP expression might indirectly involve canonical Wnt signaling (7, 8). In marked contrast, we have recently shown that in human chondrocytes IL-1β-induced Wnt/β-catenin signaling is part of a negative feedback loop inhibiting NF-κB-mediated MMP expression. In human cells β-catenin inhibits NF-κB due to a negative protein-protein interaction with p65 (9). Furthermore, also in human chondrocytes the noncanonical Wnt pathway repressed the expression of cartilage-specific extracellular matrix molecules and might be involved in chondrocyte dedifferentiation during in vitro expansion of primary chondrocytes (9, 10). At present and in marked contrast to animal models, a direct role of the canonical Wnt pathway in cartilage degeneration in human has not been identified. These findings suggest that research should be focused on human cartilage and/or human chondrocytes instead of animal models for better understanding of the role of the Wnt signaling pathway in human cartilage disease.
In this study, we have focused on the role of TCF/LEF transcription factors, the downstream effectors of Wnt/β-catenin signaling in human chondrocytes. We demonstrate that TCF4 is a pro-catabolic factor by potentiating NF-κB signaling.

EXPERIMENTAL PROCEDURES

**Human Cartilage Samples**—The collection and use of human cartilage were approved by a local medical ethical committee. Cartilage was obtained from eight patients (63 ± 10 years) with OA undergoing total knee replacement surgery. Knee cartilage was harvested from regions with no macroscopically evident degeneration. Healthy articular cartilage was obtained from three postmortem donors (66 ± 14 years) without joint diseases.

**Human Chondrocyte Isolation and Cell Culture**—Human articular chondrocytes were isolated from cartilage as described previously (10). Human chondrocytes and HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.

**Reagents**—Recombinant human IL-1β and recombinant human Wnt3A were obtained from R&D Systems.

**Plasmid Constructs and Viral Transduction**—Adenoviruses expressing null control, GFP, human TCF4, LEF1, and IκB-α (Vector Biolaboratories) were used to infect human chondrocytes at a multiplicity of infection of 100. Protein or RNA samples were harvested 72 h after adenoviral transduction unless otherwise specified.

**Human TCF3 variant1 (E12) and variant 2 (E47) ORF sequences (Origene) were cloned in to the lentiviral vector pBOB (Addgene plasmid 12335) (11). shRNA sequences against human TCF4 and LEF1 were cloned into the pLKO.1-TRC cloning vector (Addgene plasmid 10878) (12). The pLKO.1 vectors containing a scrambled shRNA (Addgene plasmid 18640) (13) were used as negative control.

The human MMP-1 (~1478 bp to +60 relative to transcription start site) and MMP-13 promoters (~1548 to +60 relative to transcription start site) were amplified by PCR using Pfu DNA polymerase (Promega) and human genomic DNA as template and cloned into pGL3-Basic vector (Promega). The promoter and/or luciferase cassette was cloned into a lentiviral vector backbone (Addgene plasmid 14715) (14). Mutations in a putative Wnt response element (WRE) in the MMP promoters were considered statistically significant.

Lentiviral vectors and packaging vectors were transfected into HEK293T cells to produce lentiviruses. Lentiviruses were harvested and used to infect chondrocytes in the presence of 6 μg/ml Polybrene (Sigma-Aldrich). Protein or RNA samples were harvested 72 h after lentiviral transduction.

**Reporter Assay**—Human chondrocytes were infected with Cignal™ lentiviruses containing TCF/LEF or NF-κB-responsive elements and luciferase reporter (SA Biosciences) together with lentiviruses constitutively expressing Renilla luciferase (SA Biosciences) in the presence of 6 μg/ml Polybrene (Sigma). Luciferase activity was measured using a Dual-Glo luciferase assay kit (Promega) 72 h after lentiviral transduction. Activity of firefly luciferase was normalized to Renilla luciferase activity.

MMP TCF/LEF promoter reporter activity and the activity of a promoterless negative control were measured using the Steady-Glo® Luciferase Assay System (Promega) 72 h after lentiviral transduction. Data were normalized for transduction efficiency by measuring luciferase DNA contents in chondrocytes using quantitative PCR (qPCR).

**RNA Isolation and Real-time RT-PCR**—Total RNA was isolated using the NucleoSpin RNA II kit (Macherey-Nagel). cDNA was synthesized from total RNA with the iScript cDNA synthesis kit (Bio-Rad). qPCR was performed using the MyiQ real-time PCR detection systems (Bio-Rad). GAPDH was used as internal control. Primer sequences are available on request.

**Immunoprecipitation and Western Blotting**—Immunoprecipitation was performed using the Dynabeads Co-Immuno-precipitation kit (Invitrogen). Total cell proteins for Western blotting were collected using radioimmunoprecipitation assay buffer (Cell Signaling) supplemented with the Halt protease and phosphatase inhibitor mixture (Thermo Scientific). Antibodies used for immunoprecipitation and Western blotting were anti-β-catenin (BD Biosciences), pro-MMP-1 (R&D Systems), pro-MMP-3 (R&D Systems), pro-MMP-13 (R&D Systems), GAPDH (Sigma-Aldrich), NF-κB p65 (Santa Cruz Biotechnology), IκB-α (Santa Cruz Biotechnology), IκB-β (Santa Cruz Biotechnology), FLAG (Origene), TCF4 (Cell Signaling), and LEF1 (Cell Signaling).

**MMP Activity Assay**—Generic MMP activity in human chondrocytes and culture media was measured using the SensoLyte 520 generic MMP activity kit (AnaSpec). Isolated proteins were incubated with 4-aminothiophenylmercuric acetate for 24 h to activate all pro-MMPs. MMP substrates were then incubated with activated proteins for 30 min, and fluorescence signals were measured. MMP activity was normalized for protein concentrations of total cell lysates measured using the Pierce BCA Protein Assay Kit (Thermo Scientific).

**TUNEL Assay**—Apoptosis of chondrocytes was detected using the DeadEnd™ Fluorometric TUNEL assay (Promega). Nuclei were counterstained with DAPI (Invitrogen).

**Caspase Activity Assay**—Caspase activity was measured using the Caspase-Glo® 3/7 Assay Systems (Promega). Caspase activity was normalized for protein concentrations measured using the Pierce BCA Protein Assay Kit (Thermo Scientific).

**Statistical Analysis**—Data were expressed as the mean ± S.D. and analyzed by two-tailed Student’s t tests. p < 0.05 was considered statistically significant.

RESULTS

**Expression of TCF/LEF Family Members in Human Articular Cartilage**—We first evaluated the mRNA expression of four TCF/LEF family members in primary human cartilage samples of osteoarthritic patients. Based on mRNA expression, TCF4 was the most abundant TCF member in human cartilage followed by TCF3 and LEF1 (Table 1). TCF1 mRNA was barely detectable.

**Effects of TCF/LEF Members on MMP mRNA Expression**—Viral transduction was used to overexpress TCF4, LEF1, and TCF3 in human chondrocytes. Adenoviral transduction of TCF4 and LEF1 significantly activated TCF/LEF reporter activity in human chondrocytes (Fig. 1A). MMP-1, -3, and -13...
**Diverse TCF/LEF Actions in Human Chondrocytes**

**TABLE 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>ΔCt</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>TCF1</td>
<td>17.67 ± 1.01</td>
<td>0.09</td>
</tr>
<tr>
<td>TCF3</td>
<td>11.01 ± 0.50</td>
<td>9.15</td>
</tr>
<tr>
<td>TCF4</td>
<td>7.74 ± 0.38</td>
<td>87.98</td>
</tr>
<tr>
<td>LEF1</td>
<td>12.72 ± 0.72</td>
<td>2.78</td>
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mRNA expression was increased by TCF4 overexpression. In contrast, LEF1 overexpression significantly decreased the mRNA expression of MMP-1, -3, and -13 (Fig. 1A). Overexpression of two TCF3 variants showed the same effect on MMP expression as LEF1 (Fig. 1B). Knockdown of TCF4 significantly decreased MMP-1 and MMP-13 mRNA expression but failed to change MMP-3 expression (Fig. 1C). Knockdown of LEF1 slightly but significantly up-regulated MMP-1 mRNA expression only (Fig. 1C).

**FIGURE 1. Effects of TCF/LEF members on MMP mRNA expression.** A, human primary chondrocytes were infected with adenoviruses expressing GFP, TCF4, and LEF1. Activation of the canonical Wnt pathway by TCF4 or LEF1 overexpression was validated by a luciferase reporter assay. *, p < 0.05; **, p < 0.01; n = 3 donors (left panel). MMP mRNA expression was measured by qPCR. *, p < 0.05; **, p < 0.01; n = 3 donors (right panel). Error bars, S.D. B, human chondrocytes were infected with lentiviruses expressing GFP, and FLAG-tagged TCF3 variant 1 (TCF3.E12) and TCF3 variant 2 (TCF3.E47). Overexpression of TCF3 was validated by Western blotting using an anti-FLAG antibody (left panel). MMP mRNA expression was measured by qPCR in human chondrocytes overexpressing TCF3 variants. ***, p < 0.01; n = 4 donors (right panel). C, human chondrocytes were infected with lentiviruses expressing scrambled shRNA (shScr) and shRNA against TCF4 or LEF1. Knockdown of TCF4 and LEF1 protein levels was validated by Western blotting (left panel). MMP mRNA expression was measured by qPCR. *, p < 0.05; **, p < 0.01; n = 3 donors (right panel).

**FIGURE 2. Effects of TCF4 and LEF1 on MMP protein expression and activity.** Human chondrocytes were infected with adenoviruses expressing GFP, TCF4, and LEF1. A, MMP protein levels were detected by Western blotting. B, generic MMP activity in culture media and cell lysates was measured using a generic MMP assay kit. ***, p < 0.01; n = 3 donors. Error bars, S.D.

**Effects of TCF4 and LEF1 on MMP Protein Expression and Activity**—In agreement with their effects on MMP mRNA expression, overexpression of TCF4 up-regulated the protein expression of MMP-1, -3, and -13 whereas LEF1 overexpression down-regulated MMP protein expression (Fig. 2A). Increased MMP protein expression by TCF4 coincided with an increase in generic MMP activity in human chondrocytes and in culture media. In contrast, LEF1 overexpression decreased generic MMP activity (Fig. 2B).

**TCF4 Potentiates NF-κB Signaling in Human Chondrocytes**—Previously, we have shown that knockdown of TCF4 in mouse cells abolished Wnt/β-catenin-induced MMP expression, indicating that at least in animal models MMPs are direct target genes of TCF/LEF transcription factors (9). To determine whether MMPs are direct target genes of TCF4 transcription factors in human chondrocytes, we analyzed 3000 bp of promoter sequence of the MMP-1 and MMP-13 genes for the presence of consensus WREs. We identified one potential WRE in the MMP-1 promoter 506 bp upstream of the transcription start site and one in the MMP-13 promoter 1144 bp upstream of the transcription start site which matched the consensus sequence (Fig. 3A). A promoter fragment of about 1.5 kb of the human MMP-1 and MMP-13 gene was cloned in front of the luciferase reporter gene, and the putative WRE sequences were mutated. Wild-type MMP-1 and MMP-13 promoters showed ~30-fold induction of promoter activity compared with a promoter-less control. Mutation of the WREs did not influence promoter activity in human chondrocytes (Fig. 3A), suggesting that the consensus WRE is not involved in regulation of MMP expression in contrast to the knockdown of TCF4 which significantly decreased MMP-1 and MMP-13 expression (Fig. 1C). However, we cannot exclude the possible existence of functional WREs in the MMP genes outside of the analyzed promoter region.

We previously showed that Wnt-3A decreased MMP expression through an inhibitory interaction of β-catenin with NF-κB p65/RELA in human chondrocytes (9). Therefore, we tested whether TCF4 might also influence NF-κB activity. Overexpression of TCF4 in human chondrocytes significantly increased both basal and IL-1β-induced NF-κB reporter activities, whereas overexpression of LEF1 slightly but significantly decreased NF-κB activities (Fig. 3B). These data suggested that TCF4 might up-regulate MMP expression by potentiating...
NF-κB signaling rather than through its conventional function in the canonical Wnt pathway. Interestingly, as shown in Fig. 3C, TCF4 co-immunoprecipitated with NF-κB p65, a key transcription factor in the regulation of MMP expression in human chondrocytes. Overexpression of TCF4 enhanced its binding to p65. LEF1 also co-precipitated with NF-κB p65 as reported previously, and this was increased by overexpression of LEF1 (Fig. 3C) (15). In agreement with previous findings, β-catenin, IκB-α, and IκB-β also co-immunoprecipitated with p65. Interestingly, overexpression of TCF4 slightly reduced co-immunoprecipitation of β-catenin, IκB-α, and IκB-β, which are all inhibitors of NF-κB. Overexpression of LEF1 decreased the binding of both TCF4 and β-catenin to p65 (Fig. 3C). Because β-catenin is an inhibitor of NF-κB (9), the repression of NF-κB activity by LEF1 may be caused by competition between LEF1 and TCF4 for binding to p65. LEF1 overexpression did not change the binding of the NF-κB inhibitors IκB-α and IκB-β to NF-κB p65, although the basal expression levels seemed to be increased by LEF1 overexpression (Fig. 3C). None of the above-mentioned proteins were detected in Western blots when control IgG was used as bait in co-immunoprecipitation assay (data not shown).

We next evaluated whether TCF4 and LEF1 could also influence the expression of other NF-κB target genes such as IL-6 and SERPINA1. IL-6 mRNA expression was significantly up-regulated by TCF4 overexpression but repressed by LEF1 overexpression (supplemental Fig. S1). However, expression of SERPINA1 was not significantly affected by either TCF4 or LEF1 overexpression (supplemental Fig. S1). This indicated that a subset of NF-κB target genes might be selectively regulated by the interaction between NF-κB and TCF4 or LEF1 transcription factors. In addition, an evaluation of NF-κB target gene expression by TCF4 or LEF1 overexpression over time was also performed (supplemental Fig. S2). TCF4 and LEF1 showed significant effects from 24 h when the protein levels started to increase, suggesting a fast and direct effect of TCF/LEF on NF-κB activity. Although the expression of IL-1β was also changed, as a target gene of NF-κB (16), the initial up-regulation of NF-κB target gene may be dependent on the interaction of TCF4 and NF-κB. The increased IL-1β may form a positive feedback to enhance the action of NF-κB signaling as shown previously (16).

In agreement with the effect of TCF4 on the binding of IκB-α to NF-κB, overexpression IκB-α counteracts the induction of expression of target genes including IL-6 (Fig. 3D) and MMPs (supplemental Fig. S3) by TCF4 overexpression as well as IL-1β treatment. It suggests that the TCF4 enhancing effect on NF-κB activity may be at least partly dependent on its inhibitory effect on the binding of IκB-α to NF-κB. TCF4-induced IL-1β expression was also almost eliminated by IκB-α overexpression (supplemental Fig. S3), indicating that the up-regulation of IL-1β expression by TCF4 is very likely to depend on its direct potentiating effect on NF-κB. Taken together, these data suggest that the effect of TCF4 is rather dependent on its direct action on p65 activity than up-regulation of IL-1β expression.

**TCF4 Induces Human Chondrocyte Apoptosis**—Overexpression of TCF4 but not of LEF1 in primary human chondrocytes induced apoptosis as determined by a TUNEL assay (Fig. 4A). Consistent with the TUNEL assay, overexpression of TCF4 elevated caspase 3/7 activity, suggesting that its effect on chondro-
cyte apoptosis was at least partly mediated through activating of caspase 3/7 (Fig. 4B).

**TCF4 mRNA Expression Is Up-regulated in OA Cartilage**—We finally studied the mRNA expression of TCF4 in OA and healthy articular cartilage samples using qPCR. As shown in Fig. 5, mRNA expression of TCF4 was significantly elevated in OA cartilage compared with healthy human articular cartilage specimens. In agreement with this, MMP-1 and MMP-13 mRNA expression was higher in OA cartilage than in healthy cartilage, although MMP-3 expression did not show any difference (Fig. 5). This suggests that increased TCF4 expression in OA cartilage may contribute to the progression of OA by potentiating the pro-catabolic NF-κB pathway and by stimulating chondrocyte apoptosis. We further explored whether TCF4 expression was regulated by Wnt or NF-κB pathways. Neither Wnt3A nor IL-1β treatment influenced TCF4 mRNA expression in human articular chondrocytes (supplemental Fig. S4). In contrast, LEF1 expression was significantly up-regulated by both stimuli (supplemental Fig. S4), in agreement with previous reports (17, 18). This implies that other factors in the osteoarthritic environment may contribute to the increased TCF4 expression.

**DISCUSSION**

Previous animal studies have suggested a catabolic and degenerative role of the Wnt/β-catenin pathway in articular cartilage. Recently we have challenged this pro-catabolic role of Wnt/β-catenin signaling in human cartilage by revealing an unprecedented species difference in the role of canonical Wnt signaling in the expression of MMP-1, -3, and -13 (9). In human chondrocytes Wnt/β-catenin signaling is part of a negative feedback loop countering IL-1β-induced MMP expression by a noncanonical inhibitory protein-protein interaction of β-catenin with NF-κB. In marked contrast to animal chondrocytes, the downstream effectors of β-catenin TCF/LEF transcription factors are not involved in IL-1β-induced MMP expression in human chondrocytes (9). This questions the role of TCF/LEF transcription factors, the downstream effectors of β-catenin in the canonical Wnt signaling pathway, in human chondrocytes.

Among all of the catabolic factors involved in cartilage degeneration, MMPs play a crucial role in collagen and proteoglycan degradation (19–21). It has been shown that multiple pathways such as the p38, NF-κB, AP-1, MAPK, and C/EBP are involved in the transcription regulation of MMPs (22–25). We demonstrated that TCF4 was a strong activator of MMP-1, -3, and -13 mRNA expression. Knockdown of TCF4 led to a decrease in the basal transcription of MMP-1 and MMP-13 but not of MMP-3. This might be due to the usage and/or compensation of other regulatory pathways in MMP-3 transcription. Despite the fact that we identified consensus WREs in the pro-
moter regions of the MMP-1 and MMP-13 genes, mutation analysis of these WREs in MMP-1 and -13 promoters failed to show their involvement in promoter regulation. Although not conclusive, these data do suggest that the effect of TCF4 might be independent of its conventional function as canonical Wnt pathway transcription factor. We cannot, however, exclude the possibility of binding of TCF4 to other WREs in MMP genes outside of the analyzed promoter fragments. Because ectopic expression of TCF4 led to an increase in the expression at the mRNA and protein level of MMP-1, -3, and -13 and increased generic MMP activity, it was suggested that elevation of TCF4 levels in cartilage may result in increased cartilage degradation. In agreement with this, we found a higher mRNA expression level of TCF4 in OA cartilage compared with healthy cartilage providing support for a pro-catabolic role of TCF4 in OA.

Because it has been shown that in human chondrocytes MMPs are direct target genes of NF-κB signaling (9), we further explored whether the effect of TCF/LEF on MMP expression was due to a cross-talk with NF-κB. TCF4 was found to augment NF-κB reporter activity in human chondrocytes. By co-immunoprecipitation assay, we observed an unexpected interaction between TCF4 and NF-κB p65, suggesting that this interaction might be responsible for the increase in MMP expression. LEF1 also forms a complex with p65, consistent with previous findings (15). How the protein complex of TCF4 and p65 increases NF-κB activity is not clear. It might be due to modification of NF-κB and/or recruitment of other co-factors to the p65-TCF4 complex, such as CBP/p300. It is known that transcriptional activity of NF-κB can be enhanced by many co-factors such as CBP/p300 and ribosomal protein S3 (26–28). Interestingly, TCF4 contains a unique domain in the C-terminal tail which binds to CBP/p300, whereas its family members TCF3 and LEF1 lack this domain (29). In addition, the C-terminal binding protein binds to TCF4 but not to LEF1 (30). The potentiating effect of TCF4 and inhibitory effect of LEF1 and TCF3 on NF-κB might be explained by the different interactions with co-factors. For example, it is possible that TCF4 stabilizes the interaction between NF-κB and its positive co-factors such as CBP/p300 and ribosomal protein S3 (26–28). Alternatively, TCF4 overexpression may reduce the binding of β-catenin, IκB-α, and IκB-β to p65, which are known negative regulators of NF-κB (9, 31). In agreement with this, IκB-α overexpression was found to counteract the positive effect of TCF4 on NF-κB target gene expression. This also supports that TCF4 probably functions through interaction with NF-κB to regulate NF-κB target gene expression. However, it remains elusive whether integration of TCF4 into the transcription complex of NF-κB is required for TCF4 regulation of NF-κB target gene expression.

In marked contrast to TCF4, LEF1 is a negative regulator of NF-κB. It is likely that LEF1 negatively regulates NF-κB activity by competing with TCF4 for binding to NF-κB p65 thereby countering the TCF4 potentiating effect on NF-κB. This is based on our observation that overexpression of LEF1 decreased the binding of TCF4 to NF-κB p65, although a direct negative effect from LEF1 cannot be excluded. Vice versa, TCF4 overexpression decreased the binding of LEF1 to NF-κB p65. The potentiating effect of TCF4 or the inhibiting effect of LEF1 on NF-κB-mediated gene transcription was not limited to MMPs but was also found for other established target genes such as IL-6. Remarkably, TCF4 or LEF1 could not affect the expression of the NF-κB target gene SERPINA1. This might be explained by the involvement of different co-factors which might not be affected by TCF/LEF (31).

We showed previously that β-catenin interacts with and inhibits NF-κB in human chondrocytes (9). It is not clear whether or how TCF/LEF members interact with NF-κB in cooperation with β-catenin. Decrease in NF-κB-associated β-catenin levels by overexpression of TCF4 may contribute to its effect on NF-κB activity. In contrast, although LEF1 overexpression weakened the interaction of NF-κB p65 with its inhibitor β-catenin, NF-κB activity was not increased by LEF1, possibly due to competition between LEF1 and TCF4 to NF-κB p65 binding. It remains to be elucidated whether the effect of TCF/LEF on NF-κB is dependent on its interaction with β-catenin.

In addition to its pro-catabolic effects, it has been suggested that NF-κB may play a role in chondrocyte apoptosis (32–34). A number of studies have described NF-κB involvement in apoptotic events in articular chondrocytes. For example, it has been shown that NF-κB activation mediates the apoptotic effect of nitric oxide in articular chondrocytes, by activating caspase 3-induced apoptosis through activation of p53 (33, 34). In our study, we found that overexpression of TCF4 induced chondrocyte apoptosis. Because TCF4 is an enhancer of NF-κB activity, the effect of TCF4 on apoptosis might be at least partly due to its potentiating effect on NF-κB signaling. This is also supported by the fact that TCF4 overexpression activates caspase 3/7, which are prime mediators of NF-κB-induced apoptosis.

Our results indicate that ectopic expression of TCF4 in human chondrocytes induces cartilage catabolism by increasing MMP expression and activity and by inducing apoptosis. We provide evidence that this action of TCF4 is independent of its function as canonical Wnt pathway transcription factor, but instead is due to a potentiating interaction with NF-κB. This is in marked contrast to the inhibitory effect of β-catenin on NF-κB activity as described previously (9). Our findings suggest that TCF4 might be a pathogenic factor in human cartilage degeneration, which is further supported by an up-regulation of TCF4 mRNA expression in OA cartilage. Therefore, targeting TCF4 activity and/or expression might be a promising avenue for the treatment of degenerative cartilage disease.

Acknowledgment—We thank Dr. Simon Mastbergen (Utrecht University, The Netherlands) for providing healthy human articular cartilage samples.

REFERENCES
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