Korean J Biol Sci 4: 389-394, 2000

Transcriptional Regulation and Apoptosis Induction by Tcf/β-Catenin Complex in Various T-Cells

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Key Words:
Tcf-1
Tcf-4
β-catenin
Transcription
Apoptosis
T-cell lines
HEK293 cell

The Tcf-1 (T-cell factor-1) protein binds to the T-cell specific enhancer sequences and plays an architectural role in the assembly of transcriptional machinery. One of the Tcf family proteins, Tcf-4, was found to be an important regulator for colon cancer development where it activates specific genes upon binding to β-catenin following Wnt signaling. We were interested in the transcriptional regulatory activities of Tcf-1 and Tcf-4 proteins in T-cells and colon cancer cells. Transactivation assay was developed using a reporter plasmid containing luciferase gene under the control of Tcf responsive elements. Luciferase activity was determined following co-transfection of the reporter along with Tcf-1 and/or β-catenin expressing plasmids. Transcription was significantly induced by β-catenin expression in all cells. Tcf-1 by itself did not induce transcription in the mature T-cell lines, but overexpressed Tcf-1 greatly activated transcription in the immature T-cell line. In addition, transfected β-catenin induced apoptosis, but co-transfected Tcf-1 suppressed apoptosis in HEK293 cells. These results suggest that Tcf-1 and β-catenin differently regulate transcription and apoptosis.

The TCF-1 (T-Cell Factor-1) protein was originally identified as a T-cell specific transcription factor that binds to the specific sequences of CD3-ε enhancers through the HMG-1 (High Mobility Group-1) DNA binding domain (van de Wetering et al., 1991). It was later found that it also binds to the moderately degenerate heptamer motif (A/T)(A/T)CAAAG in other T-cell specific genes such as TCR-α and CD4 genes. Since its target genes, TCR-α, CD4 and CD3-ε, are known to be important for T-cell differentiation and activation, it would be reasonable to expect that the TCF-1 transcription factor might have a great impact on the regulation of T-cell activities. Even though Tcf-1 expression is widely distributed in the embryo, its expression is limited to immature and mature T-cells after birth (Oosterwegel et al., 1993). Especially, its expression seems to be regulated during T-cell maturation, because a high level of Tcf-1 mRNA is found in immature single positive cells but declines thereafter (Verbeek et al., 1995). A study on the Tcf-1 knockout mice demonstrates that targeted disruption of the Tcf-1 gene accumulates immature single positive stage of thymocytes in thymus. However, such a block of T-cell maturation seems to be imperfect. It requires dual knockouts of Tcf-1 and closely related Lef-1 (Lymphoid enhancer-binding factor) for marked impairment of T-cell differentiation and gene expression (Verbeek et al., 1995; Okamura et al., 1998). Even though the exact role of Tcf-1 in T-cell maturation is not understood, there is some evidence for critical involvement of Tcf-1 in proliferation of early stage thymocytes and maintenance of stem cells (Schilham et al., 1998).

Tcf-1 is also known to be an important regulator for transcription of T-cell specific genes, especially TCR-α gene enhancer. Sequence analysis of Tcf-1 and Lef-1 showed the presence of a single HMG-1 box sequence, which is known to mediate sequence specific DNA binding in various proteins. The proteins containing HMG domains bind DNA in an unusual way; it interacts with the minor groove and induces strong bending in the DNA double helix. In fact, by structural analysis of Lef-1 on the TCR-α enhancer as a model, it is shown that it comprises a multi-protein complex and plays pivotal role in regulating chromatin structure (Hernandez-Munain et al., 1998). Thus, it seems to play an architectural role in the assembly and function of regulatory nucleoprotein complexes in the enhancers of its target genes (Love et al, 1995). However, transcriptional role of Tcf-1 is not fully understood, because its target genes have not been definitely demonstrated in T-cells. Binding of Tcf-1 in the enhancer of TCR-α gene is even implicated for developmental regulation of VDJ recombination (Roberts et al., 1997).

Various Tcf homologous proteins were identified and their signal transduction pathways emerged as an important regulator in early development and carcino-

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genesis (Clevers and van de Wetering, 1997; Morin, 1999). Especially, Tcf-3 and Tcf-4 proteins were identified as a binding partner for β-catenin protein in nucleus that acts as a critical transcriptional regulator upon activation (Behrens et al., 1996; Lee et al., 1999). In some colon cancer cells, mutant β-catenin that has defects in specific phosphorylation site by GSK-β was shown to be resistant to ubiquitin-mediated proteolysis, translocated into nucleus and formed complex with Tcf-4 proteins. β-catenin proteins, thus, behaved as an oncogene and its binding to Tcf proteins could be a critical event in the cancer development (Korinek et al., 1997; Morin et al., 1997; Rubinfeld et al., 1997). In fact, Tcf/β-catenin complex was suggested to be a transcriptional regulator of c-myc and cyclin D1 genes (He et al., 1998; Tetsu and McCormick, 1999). Such findings caused enormous excitement in cancer research field as well as in the signal transduction field. This is because Tcf proteins were actually demonstrated to act downstream of the Wnt signal pathway via β-catenin binding and the long-sought mechanisms of c-myc activation and cell cycle regulation began to surface. The Wnt signal pathway was previously known to be a critical mechanism for early development and pattern formation in various organisms, such as Drosophila, Xenopus and mouse. In fact, Lef-1 is also reported to be associated with β-catenin and to modulate transcription in response to Wnt signaling (Hsu et al., 1998). Therefore, Tcf family proteins were likely to be involved not only in early development and carcinogenesis, but also in similar pathways of critical cellular events, such as cell fate determination and differentiation, proliferation and/or apoptosis. Tcf family proteins seem to regulate these critical cellular events by binding to various proteins, not only to activators like β-catenin, but also to repressors like Groucho or CBP (Cavallo et al., 1998; Waltzer and Bienz, 1998).

We have previously demonstrated that the expression of Tcf-1 is down-regulated upon induction of T-cell apoptosis, thus it might be a key regulatory mechanism of CD3 and TCR down-regulation in the activation induced cell death of T-cells (Jeong et al., 1998; Jeon et al., 1998). These findings stimulated us to study the mechanism of Tcf-mediated transcription and its effect on apoptosis, specifically asking whether the Wnt pathway is involved also in T-cells via transcription by Tcf/β-catenin complex. In this paper, we show that the Tcf-mediated transcription is differentially regulated in immature T-cell lines as compared to mature T-cell lines. It also suggests that signal transduction pathways other than the β-catenin-mediated Wnt pathway might be operating in T-cells. In addition, apoptosis is induced by over-expressed β-catenin, but is reduced by co-expressed Tcf-1, suggesting activating and suppressing roles of β-catenin and Tcf-1 in apoptosis, respectively.

Materials and Methods

Cell lines, plasmids and reagents

Human embryonic kidney 293 cells, mouse mature T-cell EL4, and mouse glucocorticoid sensitive immature T-cell line S49.1 cells were cultured in Dulbecco modified Eagle medium, which is supplemented with 10% heat inactivated fetal bovine serum and antibiotics (100 μg/ml of streptomycin and 100 units/ml of penicillin). Jurkat cells were cultured in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum, and maintained at 37℃ in 95% air and 5% CO_2. The Tcf-1 expressing plasmid pcDNA3.1-Tcf1 contains PCR amplified whole coding region of mouse Tcf-1 in the EcoRI site of the pcDNA3.1 vector (constructed by Young-Mee Kim). Mouse Tcf-4 expressing pDH105-Tcf4b, wild-type Tcf responsive element regulated luciferase reporter plasmid pGL3-OT and mutant Tcf binding site containing pGL3-OF were kindly provided by Dr. Shivdasani in Dana Farber Institute of Harvard Medical School. The β-catenin expressing pCAN-β-catenin was kindly provided by Dr. McCrea in University of Texas. Renilla luciferase internal control pRL-TK was from dual luciferase kit (Promega). Oligonucleotides were synthesized by Bioneer Company.

Transfection and luciferase assay

293 cells were seeded at $3x10^6$ cells/ml in 100 mm polystyrene tissue culture dish and cultured for 48 h before transfection. Cells were transfected with a luciferase reporter plasmid containing three optimal Tcf binding site upstream of the pGL3 promoter (2 μg pGL3-OT), or a luciferase reporter plasmid containing three mutations in Tcf site upstream of the pGL3 promoter (2 μg pGL3-OF). 5 μg of Tcf-1 expression plasmid, 5 μg of Tcf-4 expression plasmid and/or β -catenin expression plasmid were also co-transfected using calcium phosphate transfection protocol. Luciferase activity was detected 48 h after transfection using dual luciferase kit (Promega). Jurkat, EL4, S49.1 cells were seeded at $5x10^5$ cells/ml and incubated for 24 h and 10^7 cells were used for electroporation. Cells were sometimes transfected using DMRIE-C (Gibco BRL) with 1 μg of pGL3-OT, 0.5 μg renilla luciferase internal control plasmid (pRL-TK), 1 μg of Tcf-1 expression plasmid or 1 μg of β-catenin expression plasmid. Renilla and firefly luciferase activities were detected 24 h or 48 h after transfection using dual luciferase system (Promega).

RT-PCR analysis

Total RNA was isolated from various cells and quantified by UV spectrophotometer (Bio-Rad). Two μg of RNA was reverse transcribed either using random nucleotides or using specific primers for the targeted genes (Tcf-1 primer was started from 477 nucleotide of the gene, 5'-acagaagcttgaagtttgtccgggaaaag-3' containing HindIII site; Tcf-4 primer is from 446 nucleotide of the gene, 5'-gttctcgagtgattcattgaccag-3 containing Xho I site;

β-catenin primer is from 2560 nucleotide from the start of the gene 5'-tgggatccaggacagtctaaaaccaggc-3' containing BamH I site). Random primers were used when RT-PCR with 18S rRNA was performed as well as with target genes. One tenth of the RT products was used for PCR reaction, which ran 30 cycles of denaturation at 95℃, annealing at 55℃ and polymerization at 72℃. The pairs of PCR primers are as follows: 5' Tcf-1 primers from 187 nucleotide 5'-acagaagcttgaagtttgt ccgggaaaag-3' containing EcoR I site; 3' Tcf-1 primers from 477 nucleotide 5'-acagaagcttgaagtttgtccgggaaaag-3' containing HindIII site; 5' Tcf-4 primers from 293 nucleotide 5'-gttctcgagtgattcattgaccag-3' containing EcoR I site; 3' Tcf-4 primers from 446 nucleotide 5'-gttctcgagtga ttcattgaccag-3 containing Xho I site; 5' β-catenin primer from 215 nucleotide 5'-tgggatccaggacagtctaaaaccaggc-3' containing Sac I site; 3' β-catenin primer from 2560 nucleotide 5'-tgggatccaggacagtctaaaaccaggc-3' containing BamH I site.

DNA fragmentation assay

293 cells were harvested after transfection with Tcf-1 and/or β-catenin by centrifugation by 4,000 rpm for 5 min in 4℃. 20 μl of lysis buffer was added, and the cells were resuspended and incubated at 50℃ for 1 h. One μl of 5 mg/ml RNase A was added and the reaction was continued at 50℃ for 30 min. Five μg of DNA was loaded to each lane of 2% agarose gel and electrophoresed at 80 V for 30 min.

Results

Expression of endogenous Tcf-1, Tcf-4 and β-catenin in various T-cell and B-cell lines

To understand the mechanism underlying the Tcf-mediated transcription, endogenous Tcf-1 and Tcf-4 mRNA levels were measured by RT-PCR analysis. As shown in Fig. 1A, the mature T-cell line EL4 has a low level of Tcf-1 (lanes 1 and 2) as compared to the immature T-cell line S49.1 (lanes 4 and 5). The endogenous level of 18S rRNA in each sample was also determined by the same RT-PCR condition to normalize the target message level (data not shown). This finding is consistent with the northern blot analysis of various stages of immature thymocytes and mature T-cells from mouse, suggesting that higher level of Tcf-1 is expressed in immature cells than in mature cells (Verbeek et al., 1995). We have also observed a high level of Tcf-1 in thymocytes by northern blot analysis (Jeong et al., 1998; Jeon et al., 1998). RT-PCR analysis of B-cell line BJAB demonstrates that Tcf-1 is not expressed in B-cell, as expected from the fact that Tcf-1 expression is restricted in T-cell lineage (lane 7). In contrast to Tcf-1, Tcf-4 was highly expressed in T-cell and B-cell lines as shown in lanes 3 (EL4), 6 (S49.1) and 8 (BJAB). Expression of β-catenin was not detected by RT-PCR analysis in EL4 and S49.1 cells (lanes 1 and 2), but a relatively high level was detected in BJAB (lane 3 in Fig. 1B).

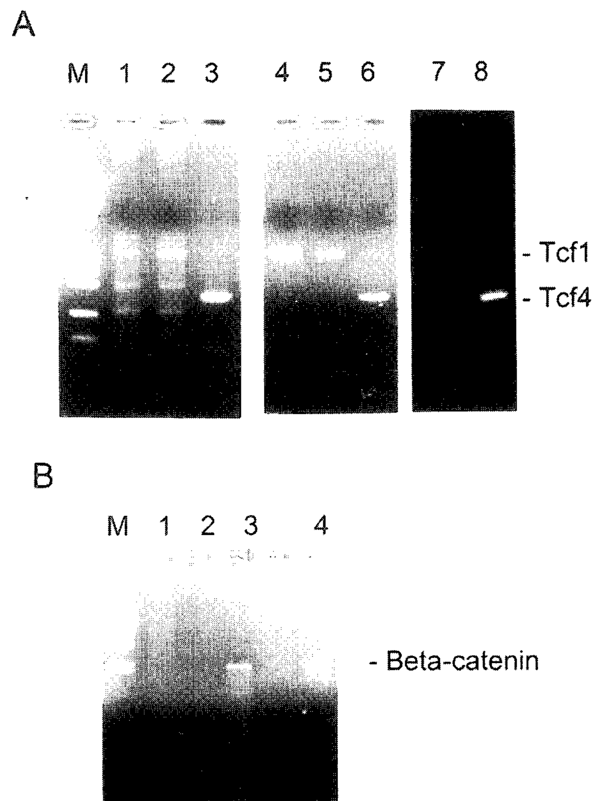

Fig. 1. Expression of endogenous Tcf-1, Tcf-4 and β-catenin genes in various cells. A, RT-PCR analysis of EL4 (lanes 1-3), S49.1 (lanes 4-6) and BJAB (lanes 7-8) total RNA was performed with Tcf-1 specific primers (lanes 1,2,4,5 and 7). Duplicate reactions were performed for EL4 (lanes 1 and 2) and S49.1 (lanes 4 and 5). Tcf-4 specific primers (lanes 3, 6 and 8) were also used to detect endogenous Tcf-4 mRNA. In each PCR reaction, internal control reaction with 18S rRNA primers was used (not shown). B, RT-PCR analysis of β-catenin mRNA from EL4 (lane 1), S49.1 (lane 2) and BJAB (lane 3). Control PCR reaction was also performed with pCAN-β-catenin plasmid DNA (lane 4).

Multiple experiments were performed to confirm β-catenin expression in these cells, but was never detected in T-cell lines at any level. It might be due to the inactivation of Wnt signaling in these cell lines.

Transcriptional regulation by Tcf/β-catenin complex in immature and mature T-cell lines

In order to establish an assay for transcriptional activation by Tcf/β-catenin complex, we utilized the reporter pGL3-OT plasmid that has the luciferase gene under the control of three copies of Tcf-responsive and control elements. The Tcf-1 and β-catenin expressing plasmids were co-transfected with pGL3-OT reporter and transactivation of luciferase reporter gene was measured at 48 h after transfection. As shown in Fig. 2, the exogenously expressed β-catenin transactivated luciferase reporter gene and resulted in two- to three-fold induction of transcription in the human mature T-cell lymphoma Jurkat and the mouse mature T-lymphoma EL4. This suggests that β-catenin alone is sufficient to activate Tcf-responsive element with endogenous Tcf-4

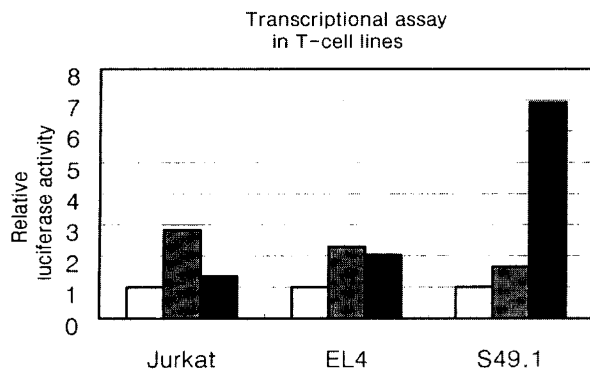

Fig. 2. Transcriptional activation of Tcf-1/β-catenin complex in various cells. Cells were transfected with the luciferase reporter pGL3-OT whose expression is under the control of the Tcf-responsive element(□). Induction of transcription by exogenously expressed genes was measured at 48 h after co-transfection of β-catenin(□) and/or Tcf-1(■) expressing plasmids. Relative luciferase activities were presented here in comparison to the value obtained from pGL3-OT alone transfected cells. Specificity of Tcf- mediated transcription was confirmed by pGL3-OF luciferase reporter plasmids containing a mutant version of Tcf-responsive element. In each transfection, reporter firefly luciferase activity was normalized by co-transfecting internal control renilla luciferase plasmid. At least three to five independent experiments were performed for each cell line.

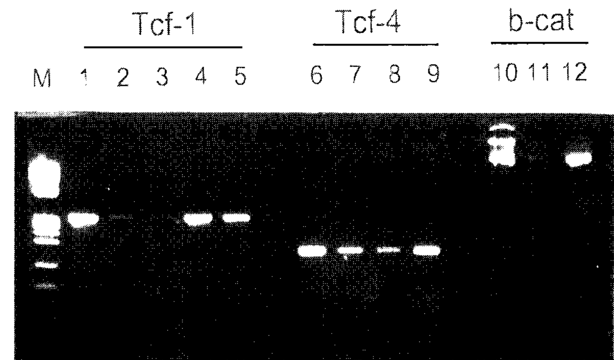

Fig. 3. Expression of transiently transfected Tcf-1, Tcf-4(A) and β-catenin(B) genes in 293 cells. RT-PCR analysis was performed with Tcf-1 specific primers (lanes 1-5), Tcf-4 specific primers (lanes 6-9) and β-catenin specific primers (lanes 10-12). Lanes 3, 7 and 11; 293 cells transfected with pcDNA3.1 vector, Lanes 4 and 8; 293 cells transfected with pcDNA3.1-Tcf1. Lanes 5 and 9; 293 cells transfected with pDH105-Tcf4b. Lane 12; 293 cells transfected with pCAN-β-catenin. In each set of RT-PCR, plasmid DNA was also amplified as a PCR control (lane 1, PCR of pcDNA3.1-Tcf1; lane 6, pDH105-Tcf4b; lane 10, pCAN-β-catenin). In lane 2, RT-PCR was performed with total RNA from pcDNA3.1 transfected 293T cell without reverse transcriptase treatment. M; Marker.

and/or Tcf-1 in mature T-cell lines, as shown in Fig. 1. However, co-transfection of Tcf-1 gene along with β-catenin slightly reduces transcription in Jurkat and EL4. These results are consistent with the previous report that Tcf-1 acts as a repressor for Tcf-4/β-catenin mediated transcription in colon cancer cells (Roose et al., 1999).

In contrast to mature T-cell lymphoma, β-catenin alone did not significantly increase the luciferase activity in the glucocorticoid-sensitive immature T-cell thymoma line S49.1 where endogenous levels of Tcf-1 and Tcf-4 mRNAs are both high. This might result from the competition of Tcf-1 and Tcf4 for binding to β-catenin and potential repressive role of Tcf-1 in transcription. However, the exogenously over-expressed Tcf-1 plays dramatically different roles in immature cell and fully activates luciferase activity in transient transfection assay in S49.1. It is likely that a high level of Tcf-1 acts either as an activator of transcription by binding to β-catenin, or as a recruiter of proteins other than β-catenin, activating β-catenin-independent pathways in immature T-cells.

Transcriptional activation of Tcf/β-catenin complex in 293 cells and its effect on apoptosis

To study the roles of Tcf and/or β-catenin in transfection-competent cell lines, we used human embryonic kidney 293 cells to set up a transcriptional reporter assay and an apoptosis assay. 293 cells were transiently transfected with Tcf-1, Tcf-4 and/or β-catenin expressing plasmids and their expression levels were analyzed by RT-PCR (Fig. 3). There were no detectable endogenous Tcf-1 (lane 3) and β-catenin mRNA (lane 11), but a relatively high level of Tcf-4 mRNA (lane 7) in 293 cells. Expression of the transfected genes were also comfirmed as expected (lane 4, Tcf-1; lane 9, Tcf-4; lane 12, β-catenin). Interestingly, over-expression of the transfected Tcf-4 gene induced endogenous Tcf-1 mRNA (lane 5) at a relatively high level, whereas the transfected Tcf-1 gene greatly reduced endogenous Tcf-4 mRNA level (lane 8). Not only this finding is consistent with the previous report that the Tcf-1 might be a target for Tcf-4 (Roose et al., 1999), but also it suggests that Tcf-1 is a negative regulator of Tcf-4. It will be interesting to directly test for such feedback regulation of Tcf-1 and Tcf-4 genes in real cellular context.

Transcripitional activation of Tcf-1/β-catenin complex was also tested using Tcf-responsive reporter pGL3-OT (Fig. 4A), as described above. In 293 cells, transcriptional activation by Tcf-1/β-catenin was much higher than in T-cells, which is likely due to higher transfection efficiency and expression levels of transfected genes. Transcription was greatly activated by β-catenin alone, which probably resulted from endogenous Tcf-4/exogenous β-catenin complex acting as activators. Transfected Tcf-1 also induced transcription but at a much lower level than β-catenin did, which is likely from the fact that β-catenin is not expressed in the Tcf-1 transfected 293 cells. Some level of activation by exogenous Tcf-1, however, suggests that Tcf-1 might bind to other proteins in the cells and operate as part of other pathways, but with much lower transcriptional activation than the Wnt/β-catenin pathway does. Co-expression of Tcf-1 and β-catenin also increased transcription at a high level, but not much higher than expression of β-catenin alone did. This suggests that β-catenin is a critical component of Tcf-mediated transcription, as previously suggested.

We next asked whether the Tcf/β-catenin complex has any effect other than the direct transcription on target genes. Since we have previously observed reduction in Tcf-1 expression level after apoptosis induction

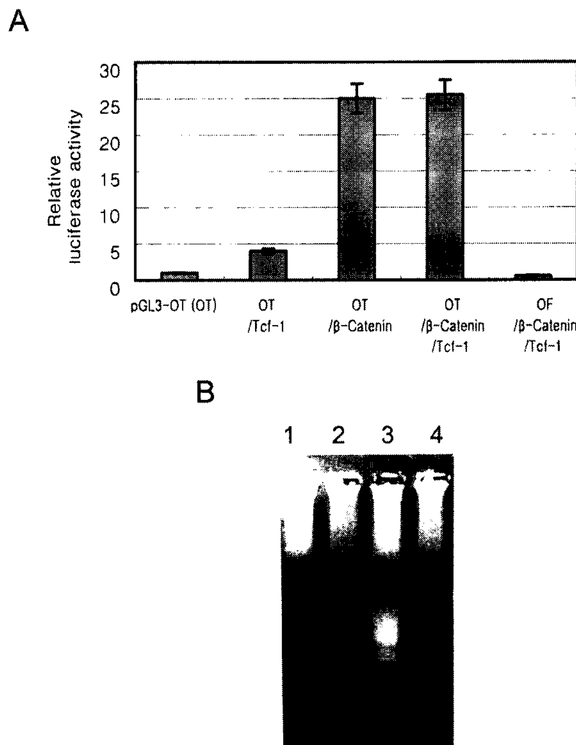

Fig. 4. Effect of transfected Tcf-1, Tcf-4 and β-catenin genes in 293 cells. A, Transcriptional assay was performed by co-transfecting pGL3-OT reporter DNA along with expression constructs and by measuring luciferase activity that is under the control of the Tcf Responsive Element in pGL3-OT. Relative luciferase activities were presented in this figure in comparison to the value obtained from pGL3-OT alone transfection. Internal control using Renilla luciferase activity was measured and normalized for each sample. Five to six independent experiments were performed. B, DNA fragmentation assay was performed at 24 h after transfection of Tcf-1 and/or β-catenin expressing plasmids into 293 cells. Lane 1; DNA from pcDNA3.1 transfected cells, Lane 2; DNA from Tcf-1 transfected cells, Lane 3; DNA from β-catenin transfected cells, Lane 4; DNA from Tcf-1 and β-catenin co-transfected cells.

in T-cells (Jeon et al., 1998; Jeong et al., 1998), we hypothesized that Tcf-1 might act as a repressor of apoptosis. To test for this possibility, we transfected Tcf-1 and/or β-catenin harboring plasmids into 293 cells and assessed the apoptosis level by DNA fragmentation assay and acridine orange/ethidium bromide staining. As shown in Fig. 4B, DNA fragmentation was observed in β-catenin over-expressed cells (lane 3), but not in Tcf-1 alone (lane 2) or Tcf-1/β-catenin co-expressed cells (lane 4). This result is consistent with the recent finding that β-catenin over-expression leads to induction of apoptosis in NIH3T3 cells (Kim et al., 2000). Interestingly, co-expression of Tcf-1 along with β-catenin does not show any sign of apoptosis, suggesting a role of Tcf-1 as a repressor of β-catenin induced apoptosis in 293 cells. A study is underway to investigate the mechanism of Tcf/β-catenin complex in transcription and apoptosis.

Discussion

Even though the Wnt signaling is demonstrated to be critical in carcinogenesis and early development, how β-catenin/Tcf complex works in various cells is not completely understood. We were also interested whether the Wnt signaling via β-catenin is operating in T-cells and how it is regulated. In this paper, we investigated the endogenous levels of Tcf-1, Tcf-4 and β-catenin mRNA in various cell lines. Tcf-1 expression was restricted to T-lineage cell lines, but at a slightly higher level in the immature cell line, as previously demonstrated by northern blot analysis of various stages of thymocytes (Verbeek et al., 1995). In various T-cell lines, transfection of Tcf-1 or β-catenin increased the Tcf-responsive element containing luciferase reporter activity to a higher level, but co-transfection of these two genes did not increase the activation level any further. This suggests that β-catenin acts as an activator of Tcf-mediated transcription, as previously demonstrated in colon cancer cells (Korinek et al., 1997). Such activating effect of β-catenin might result from binding of exogenous β-catenin with endogenous Tcf-4, which seems to be present at a significant level in T-cell lines. Interestingly, expression of the exogenous Tcf-1 even reduced the β-catenin induced transcripion in mature T-cell lines, but greatly increased transcription in the immature T-cell line. One possible explanation for this phenomenon would be that higher level of Tcf-1 acts as a repressor in mature cells, but as an activator in immature T-cells. It is previously suggested that Tcf-1 repressed transcriptional activity of Tcf-4/β-catenin complex in cancer cell, although the exact mechanism of the repression was not understood at all (Roose et al., 1999).

It will be interesting to decipher the mechanism of the differential roles of Tcf-1 in immature and mature T-cells. Similar repression mechanism as in colon cancer cells might work in mature T-cells, but an alternative signal transduction pathway may be operating in immature T-cells. Since β-catenin did not seem to be highly expressed in various T-cell lines in the absence of activation, the Wnt signaling may not be a major pathway in T-lymphocytes. In fact, it is reported that GSK-3β inactivation that is a direct cause of β-catenin activation may not be involved in Tcf-mediated transcription in T-cells (Staal et al., 1999). We observed that co-expression of β-catenin and Tcf-1 greatly induced transcription level, as compared to the transfection of β-catenin alone did, in S49.1 cells. This suggests that Tcf-1 might activate transcription via activators other than β-catenin, especially in immature T-cells. It has been recently reported that MAPK related pathway negatively regulates Tcf/β-catenin pathway in mammalian cells and C.elegans (Ishita et al., 1999; Meneghini et al., 1999). However, a positive regulator of Tcf activity other than β-catenin has never been reported.

Since Tcf/β-catenin is shown to be involved in oncogenesis, it can be presumed that Tcf/β-catenin complex might act on transcription of genes responsible

for activation of cell proliferation or suppression of apoptosis. In fact, cyclinD1, an important regulator of cell cycle was shown to be a target of Tcf/β-catenin complex. Transfection of β-catenin induced apoptosis in 293 cells (lane 3 of Fig. 4B), whereas Tcf-1 alone or Tcf-1/β-catenin co-transfection did not (lanes 2 and 4 in Fig. 4B). It is interesting that apoptosis is suppressed by the overexpressed Tcf-1, because other researchers also reported that apoptosis is induced by β-catenin and it might be involved in apoptotic process (Fukuda, 1999; Kim et al., 2000). Considering the reduction of Tcf-1 mRNA upon apoptosis induction in T-cells as we previously reported, it is likely that Tcf-1 is involved in suppression of apoptosis either by directly suppressing apoptotic activity of β-catenin, or by indirectly regulating transcription of genes responsible for apoptosis suppression. Further studies are needed to test these possibilities, especially on the regulatory mechanism of Tcf/β-catenin complex on the activating or suppressing apoptotic genes.

Acknowledgement

This study was financially supported by Korea Research Foundation to SJ (KRF-1997-019-D00212).

References

Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, and Birchmeier W (1996) Functional interaction of β-catenin with the transcription factor LEF-1. *Nature* 382: 638.

Cavallo RA, Cox RT, Moline MM, Roose J, Polevoy GA, Clevers H, Peifer M, and Bejsovec A (1998) *Drosophila* Tcf and Groucho interact to repress wingless signalling activity. *Nature* 395: 604-612.

Clevers H and van de Wetering M (1997) TCF earn their wings. *Trends Genet* 13: 485-489.

Fukuda K (1999) Apoptosis-associated cleavage of β-catenin in human colon cancer and rat hepatoma cells. *Int J Biochem Cell Biol* 31: 519-529.

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, Costa LTD, Morin PJ, Vogelstein B, and Kinzler KW (1998) Identification of *c-MYC* as a target of the APC pathway. *Science* 281: 1509-1512.

Hernandez-Munain C, Roberts JL, and Krangel MS (1998) Cooperation among multiple trascription factors Is required for access to minimal T-cell receptor α-enhancer chromatin *In vivo*. *Mol Cell Biol* 18: 3223-3233.

Hsu SC, Galceran J, and Grosschedl R (1998) Modulation of transcriptional regulation by LEF-1 in response to *Wnt-1* signaling and association with β-catenin. *Mol Cell Biol* 18: 4807-4818.

Ishitani T, Ninomiya-Tsuji J, Nagai S, Nishita M, Meneghini M, Barkers N, Waterman M, Bowerman B, Clevers H, Shibuya H, and Matsumoto K (1999) The TAK1-NLK-MAPK-related pathway antagonizes signaling between β-catenin and transcription factor TCF. *Nature* 399: 798-802.

Jeon SH, Jeong SJ, Lee CJ, Kim JK, Kim YS, Chung HY, Park SD, and Seong RH (1998) Expression of *Tcf-1* mRNA and surface TCR-CD3 complexes are reduced during apoptosis of T cells. *Int Immunol* 10: 1519-1527.

Jeong SJ, Jeon SH, Yim JB, Park SD, and Seong RH (1998) Down-regulation of *Tcf-1* Expression by activation-induced apoptosis of T cell hybridoma. *Korean J Biol Sci* 2: 403-410.

Kim K, Pang KM, Evans M, and Hay ED (2000) Overexpression of beta-catenin induces apoptosis independent of its transctivation function with LEF-1 or the involvement of major G1 cell cycle. *Mol Biol Cell* 11: 3509-3523.

Korinek V, Barker N, Morin PJ, Wichen DV, Weger RD, Kinzler KW, Vogelstein B, and Clevers H (1997) Constitutive transcriptional activation by a β-catenin-Tcf complex in $APC^{-/-}$ colon carcinoma. *Science* 275: 1784-1787.

Lee YJ, Swencki B, Shoichet S, and Shivdasani R.A. (1999) A Possible role for the high mobility group box transcrition factor *Tcf-4* in vertebrate gut epithelial cell differentiation. *J Biol Chem* 274: 1566-1572.

Love JJ, Li X, Case DA, Giese K, Grosschedl R, and Wright P (1995) Structural basis for DNA bending by the architectural trascription factor LEF-1. *Nature* 376: 791-795.

Meneghini MD, Ishitani T, Carter JC, Hisamoto N, Ninomita-Tsuji J, Thorpe CJ, Hamill DR, Matsumoto K, and Bowerman B (1999) MAP kinase and *Wnt* pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* 399: 793-797.

Morin PJ (1999) β-catenin signaling and cancer. *BioEssays* 21: 1021-1030.

Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, and Kinzler KW (1997) Activation of β -catenin-Tcf signaling in colon cancer by mutation in β-catenin or APC. *Science* 275: 1787-1790.

Okamura RM, Sigvardsson M, Galceran J, verbeek S, Clevers H, and Grosschedl R (1998) Redundant regulation of T cell differentiation and *TCF-α* gene expression by trascription factors LEF-1 and TCF-1. *Immunity* 8: 11-20.

Oosterwegel M, van de Wetering M, Timmerman J, Kruisbeek A, Destree O, Meijink F, and Clevers H (1993) Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis. *Development* 118: 439-448.

Roose J, Huls G, van Beest M, Moerer P, van der Horn K, Goldchmeding R, Logtenberg T, and Clevers H (1999) Synergy between tumor suppressor APC and the β-catenin-Tcf4 target Tcf1. *Science* 285: 1923-1926.

Roberts JL, Lauzurica P, and Kragel MS (1997) Developmental regulation of VDJ recombination by the core fragment of the T cell recptor α enhancer. *J Exp Med* 185: 131-140.

Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, and Polakis P (1997) Stabilization of β-catenin by genetic defects in melanoma cell lines. *Science* 275: 1790-1792.

Schilham MW, Wilson A, Moerer P, Bemaissa-Trouw BJ, Cumano A, and Clevers HC (1998) Critical involvement of Tcf-1 in expansion of thymocytes. *J Immunol* 161: 3984-3991.

Staal FJT, Burgering MT,van de Wetering M, and Clevers HC (1999) Tcf-1-mediated transcription in T lymphocytes: differential role for glycogen synthase kinase-3 in fibroblasts and T cells. *Int Immunol* 11: 317-323.

Tetsu O and McCormick F (1999) β-Catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398: 422-426.

van de Wetering M, Oosterwegel M, Dooiges D, and Clevers H (1991) Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. *EMBO J* 10: 123-132.

Verbeek S, Izon D, Hofhuis F, Robabus-Maandag E, Riele H, Wetering M, Oosterwegel M, Wilson A, MacDonald HR, and Clevers H (1995) An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature* 374: 70-74.

Waltzer L and Bienz M (1998) *Drosophila* CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* 395: 521-525.

[Received August 18, 2000; accepted October 6, 2000]