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# Regulation of E2F-mediated Transcription by FBXL-6 in HCT116 Cells

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F-box proteins are components of Skp1p-Cullin-F-box protein (SCF) ubiquitin-ligase complexes, where they mediate the critical step of substrate recognition. Some of the F-box proteins are responsible for selective degradation of cell cycle regulators. The F-box and leucine-rich repeat protein 6 (FBXL-6), an isoform of F-box protein family, has been cloned but its functions are barely known. In this report we examined the effect of FBXL-6 on the E2F-mediated transcription in HCT116 colorectal cancer cells. First, we found through transient transfection of E2F-responsive reporter vectors that the E2F activity is significantly repressed in HCT116 cells. Forced expression of 5'-truncated FBXL-6 in these cells could help the repression relieved and increase the E2F-mediated transcriptional activity. RT-PCR analysis revealed that the FBXL-6 mRNA is expressed in most of the cancer cells at varying degrees. We then confirmed, using a full-length cDNA expression vector, that FBXL-6 could augment the E2F-mediated transcription in various other cancer cell lines originated from hepatocellular, gastric or colorectal cancers. These results suggest the possibility that FBXL-6 may regulate the activity or stability of a protein constituting the E2F pathway via a post-translational modification.

**Key Words:** E2F transcription factor, F-box protein, FBXL-6, HCT116 cells

## INTRODUCTION

E2Fs are essential transcription factors that regulate a subset of genes required for cell cycle progression and entry into the S stage.[1,2] The mammalian E2Fs are DNA-binding heterodimers composed of two discriminating protein subunits, E2F and DP, each of which is a member of respective protein family.[3,4] So far, six members of E2F family and two members of DP family have been characterized.[5] These transcription factors regulate the activity of promoters containing E2F binding sites.[1,2] The cellular genes controlled by E2Fs encode nucleotide biosynthesis enzymes, cyclin-depedent kinases, nuclear oncogenes, retinoblastoma tumor suppressor protein (pRB) family and E2Fs.[1,2,6,7] Among these

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genes, pRB was the first tumor suppressor protein to be cloned.[8] This protein is a transcriptional repressor and a member of multigene family, containing additional two related proteins, p107 and p130.[9,10] It plays important roles in regulating the cell cycle, apoptosis and differentiation and all of these activities are pertinent to its role as a tumor suppressor.[9,11] The major growth inhibitory effects of pRB are dependent on its regulation of the E2F transcription factors through a physical association with them.[1,2,9,11] pRB also interacts with a number of proteins including various cellular targets[9,12] as well as viral oncoproteins such as the papillomavirus E7 protein.[13,14]

F-box proteins were first characterized as components of Skp1p-Cdc53p (cullin)-F-box protein (SCF) ubiquitin-ligase complexes,[15,16] in which they bind substrates for ubiquitin-mediated proteolysis.[17] There are many isoforms of F-box protein in various eukaryotic organisms.[17,18] The F-box motif itself is generally found in the N-terminal half of proteins and is often coupled with other motifs in the C-terminal part of the protein, the two most common of which in human are leucine-rich repeats and WD repeats.[18] Recently, a novel member of F-box protein family named F-box and leucine-rich repeat protein 6 (FBXL-6) was cloned but the biological studies on its cellular functions, regulatory processes and expression patterns are yet to be reported.[19,20]

In this study, we investigated the effect of FBXL-6 on the activity of E2F transcription factors. We show that FBXL-6 can increase the E2F-mediated transcription in HCT116 colorectal cancer (CRC) cells. The expression of FBXL-6 mRNA was also determined in various cancer cell lines. Finally, we confirmed the FBXL-6-induced augmentation of the E2F activity in various cancer cell lines with a different tissue origin.

## MATERIALS AND METHODS

### 1) Cell culture

Human hepatocellular carcimoma (HCC) cells such as Hep3B and PLC/PRF/5, and human cervical carcinoma cells C33A were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with penicillin (100 IU/ml), streptomycin (100 $\mu$g/ml), 2.0 mM L-glutamine (Gibco BRL) and 10% fetal bovine serum (FBS, HyClone) at 37°C in an incubator flushed continuously with 5% $CO_2$. Four SNU series HCC cell lines (SNU-354, -368, -387 and -449), two SNU series gastric cancer (GC) cells (SNU-484 and -638) and six CRC cells (SW480, SW620, DLD1, HCT15, HCT116 and Colo205) were cultured in RPMI1640 supplemented with penicillin (100 IU/ml), streptomycin (100 $\mu$g/ml) and 10% FBS at 37°C in an incubator flushed continuously with 5% $CO_2$.

### 2) Vector construction

For quantitative estimation of the endogenous E2F transcriptional activities and the effect of 5'-truncated FBXL-6 on the E2F-mediated gene transcription in HCT116 cells, $\beta$-galactosidase reporter ($\beta$-Gal, LacZ) vectors were used. p(E2F)$_6$/Min-LacZ was constructed by inserting 6 copies of consensus E2F binding sites upstream of the minimal promoter of HSV-tk gene in pMin-LacZ vector. pSp1/Min-LacZ and p(E2F)$_6$/Sp1/Min-LacZ were the same as pMin-LacZ and p(E2F)$_6$/Min-LacZ except that they contained an additional binding site for Sp1 and CTF between the E2F binding sites and the minimal promoter sequence. p(E2F)$_6$/Min-Luc vector was prepared by substituting the LacZ gene in p(E2F)$_6$/Min-LacZ with the firefly luciferase gene. The expression vectors for 5'-truncated and full-length FBXL-6 were obtained from 21C Frontier Human Gene Bank (Korean UniGene number, KU002795 and KU000455, respectively).

### 3) Transient transfection

The plasmid DNAs were transfected into the cancer cells using LipofectAMINE Plus Reagent (Invitrogen) according to supplier's protocol. To measure the endogenous E2F transcriptional activities in the cancer cells, 1 $\mu$g of the E2F-responsive reporter vectors such as pMin-LacZ, p(E2F)$_6$/Min-LacZ, pSp1/Min-LacZ or p(E2F)$_6$/Sp1/Min-LacZ or the positive control vector H$\beta$A-LacZ were co-transfected with 0.2 $\mu$g of the internal control plasmid (pGL2, Promega). To determine the effect of

FBXL-6 gene on the E2F-responsive promoter activity, an expression vector for a 5'-truncated partial cDNA of the FBXL-6 gene was co-transfected with the above-mentioned E2F-responsive reporter vectors and the internal control plasmid. For confirmation of the effects of FBXL-6 on E2F-mediated gene transcription, 1 μg of the E2F-responsive luciferase vector ($p(E2F)_6$/Min-Luc) and 0.2 μg of the internal control (CMV-LacZ) were co-transfected with 1 or 2 μg of the expression vector encoding a full-length FBXL-6 cDNA sequence or 2 μg of pcDNA3.0 (Invitrogen) for the negative control. After transfection, the cells were incubated in a 5% $CO_2$ incubator for 3 hr, added with FBS to bring the final serum concentration to 10% and incubated further for 48 hr before harvesting for luciferase and β-Gal assays.

#### 4) Cell extraction and luciferase assay

Harvested cells were washed in PBS and resuspended in 50 μl of ice-cold 250 mM Tris (pH 7.8)/1 mM DTT. Cells were lysed by three freeze-thaw cycles using liquid nitrogen and a 37°C water bath. The cell lysate was centrifuged at 12,000 rpm for 15 min at 4°C to pellet debris. The luciferase activity was measured using a 10 μl aliquot of cell lysate mixed with 350 μl of solution A (25 mM glycyglycine (pH 7.8), 2 mM ATP and 10 mM $MgSO_4$). The luciferase light units were recorded for 30 sec in the integration mode in a luminometer (Berthold Lumat LB9501, Wildbad), which automatically injected 100 μl of solution B (60 μg of D-luciferin/ml of 5 mM glycyglycine (pH 7.8)).

#### 5) β-Galactosidase Assay

The β-Gal activity in the cell lysate was determined as followings. Forty microliter of the lysate was mixed with 800 μl of chilled solution I (60 mM $Na_2HPO_4$, 40 mM $NaH_2PO_4$, 10 mM KCl, 1 mM $MgCl_2$ and 50 mM β-mercaptoethanol). At 30 sec intervals, 200 μl of solution II (60 mM $Na_2HPO_4$, 40 mM $NaH_2PO_4$ and 2 mg/ml 2-nitrophenyl-β-D-galactopyranoside (ONPG, Aldrich)) was added to this mixture and, after brief vortexing, the samples were incubated at 37°C. When the reaction mixture developed appropriate level of yellow color, 500 μl of 1 M $Na_2CO_3$ was added at 30 sec intervals to stop the reaction, and the absorbance of the resulting mixture was measured at 420 nm.

#### 6) Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated according to the single-step guanidium thiocyanate-phenol-chloroform extraction procedure using Trizol (Invitogen). 5 μg of each RNA sample was converted into the corresponding cDNA using M-MLV reverse transcriptase (Promega) and oligo $(dT)_{15}$ according to the manufacture's instruction. For amplification of FBXL-6 transcripts, the following primers were used: 5'-GTACCACCTGCTGCAGTCCGACAG-3' (sense) and 5'-AGACTTCCAGTGGATGAGGGTCAG-3' (antisense). The cDNA sequence of β-actin was also amplified as a control in a similar way using the following primers: 5'-GACTACCTCATGAAGATC-3' (sense) and 5'-GATCCACATCTGCTGGAA-3' (antisense).[21)] The PCR fragments were size-fractionated by agarose-gel electrophoresis and visualized by ethidium bromide staining.

## RESULTS

#### 1) The effect of 5'-truncated FBXL-6 on the E2F-mediated gene transcription in HCT 116 cells

Before measuring the effect of FBXL-6 on the E2F-mediated gene transcription, we determined the endogenous E2F transcriptional activity in HCT116 cells using E2F-responsive reporter vectors. The E2F-responsive reporter vectors $p(E2F)_6$/Min-LacZ and $p(E2F)_6$/Sp1/Min-LacZ were derived from pMin-LacZ and pSp1/Min-LacZ, respectively, by inserting 6-mer E2F binding sites. $p(E2F)_6$/Sp1/Min-LacZ contains binding sites for universal transcription factors such as Sp1 and CTF, and thus can measure the synergism or antagonism between them and E2Fs.

The endogenous E2F transcriptional activity was estimated by calculating the reporter activity ratios, $p(E2F)_6$/Min-LacZ to pMin-LacZ and $p(E2F)_6$/Sp1/Min-

LacZ to pSp1/Min-LacZ, respectively. In HCT116 cells, these values were 0.47 and 0.29, respectively, indicating that E2Fs worked as active repressors for the transcription mediated by universal transcription factors rather than as transactivators. Therefore, in HCT116 cells the functional status of E2Fs appears to be similar to that in normal cells (Fig. 1A). Next, we estimated the E2F transcriptional activity after transfecting an expression vector encoding 5'-truncated FBXL-6 gene into HCT116 cells. Transfection of pcDNA3.0 did not significantly change the activity ratio of p(E2F)6/Min-LacZ to pMin-LacZ from the endogenous pattern (Compare Figs. 1A and 1B). However, upon expression of 5'-truncated FBXL-6, the activity ratio increased to 1.57 fold, indicating that this gene could augment the E2F-mediated transcription (Fig. 1B). Similarly, the activity ratio of p(E2F)6/Sp1/Min-LacZ to pSp1/Min-LacZ was also increased by 5'-truncated FBXL-6 (Fig. 1C). These results suggest that the 5'-truncated FBXL-6 can induce the relief of E2F from pRB-mediated check and its subsequent activation.

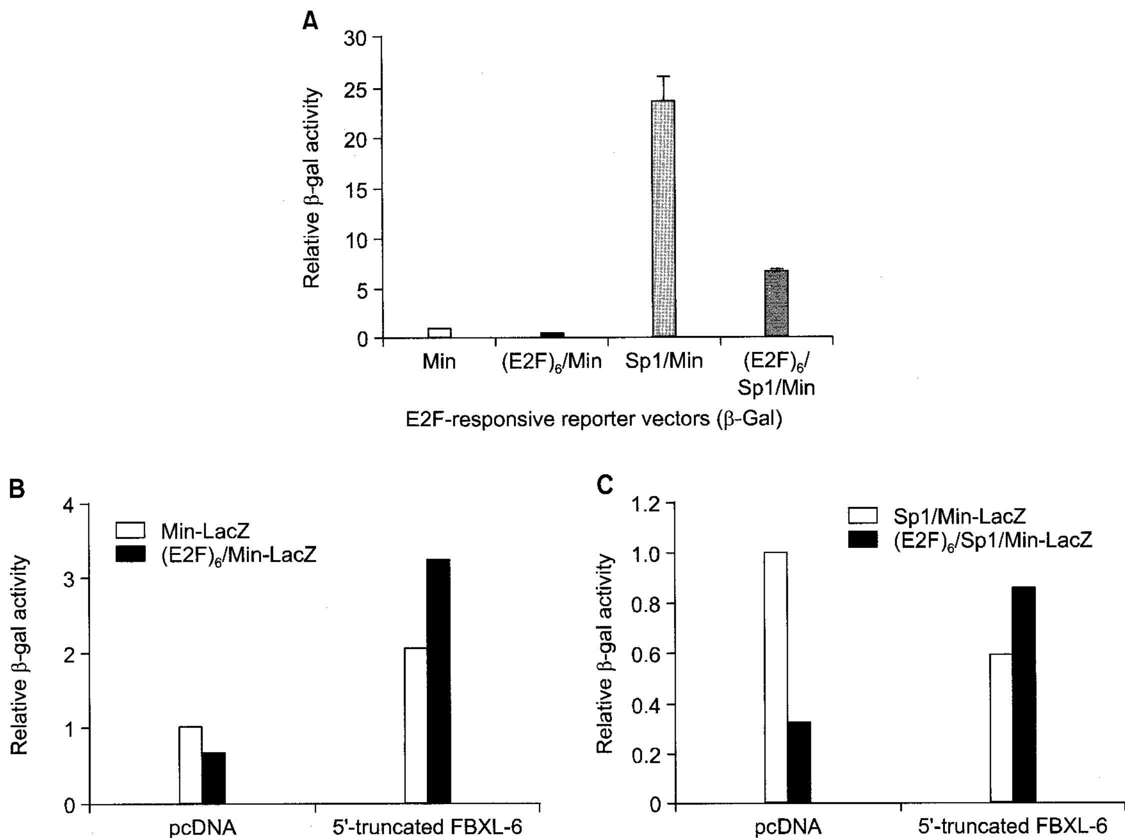


**Fig. 1.** Effects of 5'-truncated FBXL-6 on the E2F-mediated transcription in HCT116 cells. (A) To evaluate the endogenous E2F transcriptional activities, 1 $\mu$g of the E2F-responsive reporter vectors or H$\beta$A-LacZ control plasmid were introduced into HCT116 colorectal carcinoma cells. (B) and (C); To measure the effect of 5'-truncated FBXL-6 on the E2F-mediated gene transcription, 0.4 $\mu$g of 5'-truncated FBXL-6 expression vector or pcDNA3.0 were co-transfected into HCT116 cells with (B) 0.4 $\mu$g of (E2F)6/Min-LacZ or Min-LacZ reporter plasmid, or with (C) 0.4 $\mu$g of (E2F)6/Sp1/Min-LacZ or Sp1/Min-LacZ. Every transfection mixture contained 0.125 $\mu$g of internal control plasmid (pGL2-Luc). $\beta$-Gal activities in the cell lysate were measured by reading OD at 420 nm and finally normalized for the transfection efficiency. pcDNA3.0 was used as the negative control in place of the 5'-truncated FBXL-6 expression vector.

### 2) The expression pattern of FBXL-6 message in various cancer cells

In order to examine the endogenous level of FBXL-6 mRNA in various cancer cell lines, we performed RT-PCR analysis using human-specific FBXL-6 primers and total RNA extracted from each cell. As shown in Fig. 2, almost all cancer cells except for SNU-387 expressed variable levels of FBXL-6 mRNA. It was relatively highly expressed in Hep3B, SNU-449, HCT116, SW480, SW620 and DLD1 cells, while only weakly or rarely in many other cell lines. This result indicates that the expression pattern of FBXL-6 mRNA can be represented diversely in different cell types. Since E2Fs are ubiquitously expressed, the universal expression pattern of FBXL-6 mRNA suggests that this gene might be intimately involved in the regulation of cell cycle progression by modulating the E2F activities.

### 3) Confirmation of the FBXL-6-induced augmentation of E2F activity in various cancer cells

As mentioned above, we showed that 5'-truncated FBXL-6 could augment the E2F-mediated gene transcription in HCT116 cells. We then examined the generality and the dose-dependence of this effect by introducing two different amounts of the full-length expression vector for FBXL-6 gene into various cancer cells. In HCT116 CRC cells, the full-length FBXL-6 increased the E2F-mediated transcriptional activity in a dose-dependent manner (Fig. 3A). We also observed similar phenomena in HCC cells such as SNU-354 (Fig. 3B), -368, -387, and -449 (data not shown), and GC cells including SNU-484 (Fig. 3C) and -638 (data not shown). These results indicate that the FBXL-6-induced augmentation of the E2F activity is a general phenomenon, and suggest the possibility that FBXL-6 may regulate the activity or stability of a protein constituting the E2F pathway via a post-translational modification.

## DISCUSSION

Ubiquitin-dependent proteolysis plays key roles in the control of many vital processes, including cell-cycle progression, signal transduction, transcriptional regulation, receptor down-regulation, immune response and development.[22] In the ubiquitin system, many proteins are targeted for degradation by covalent ligation to ubiquitin, a highly conserved 76 amino acid protein.[23] This protein is activated in a thioester linkage to a ubiquitin-activating enzyme (E1), then transferred to a ubiquitin-conjugating enzyme (E2) and, in conjunction with a ubiquitin-protein ligase (E3), ultimately conjugated to a lysine residue of a substrate protein in an isopeptide linkage.[17,22] Especially, E3 ubiquitin-protein ligases mediate the critical step of diverse substrate recognition, and there are numerous potential E3 ligases in the human genome.[24]

F-box proteins are components of SCF protein complex, one class of E3 ubiquitin protein ligases.[15,16,24]

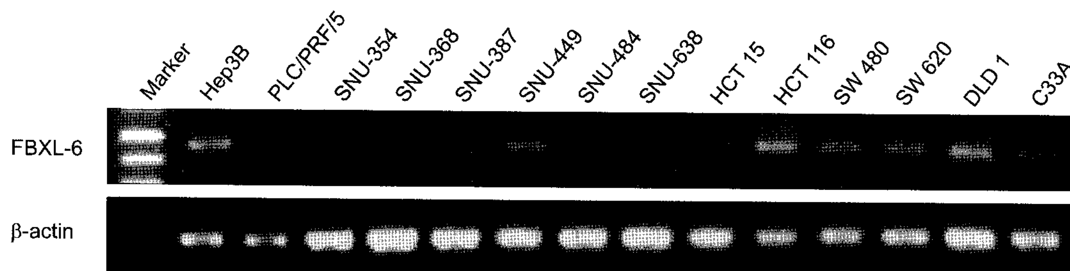


**Fig. 2.** Expression of FBXL-6 mRNAs in various cancer cells. RT-PCR was performed with human specific primers for FBXL-6 using 5 $\mu$g of total RNA from various cancer cell lines. $\beta$-Actin specific primers were used for the control. One-kb size markers are shown.

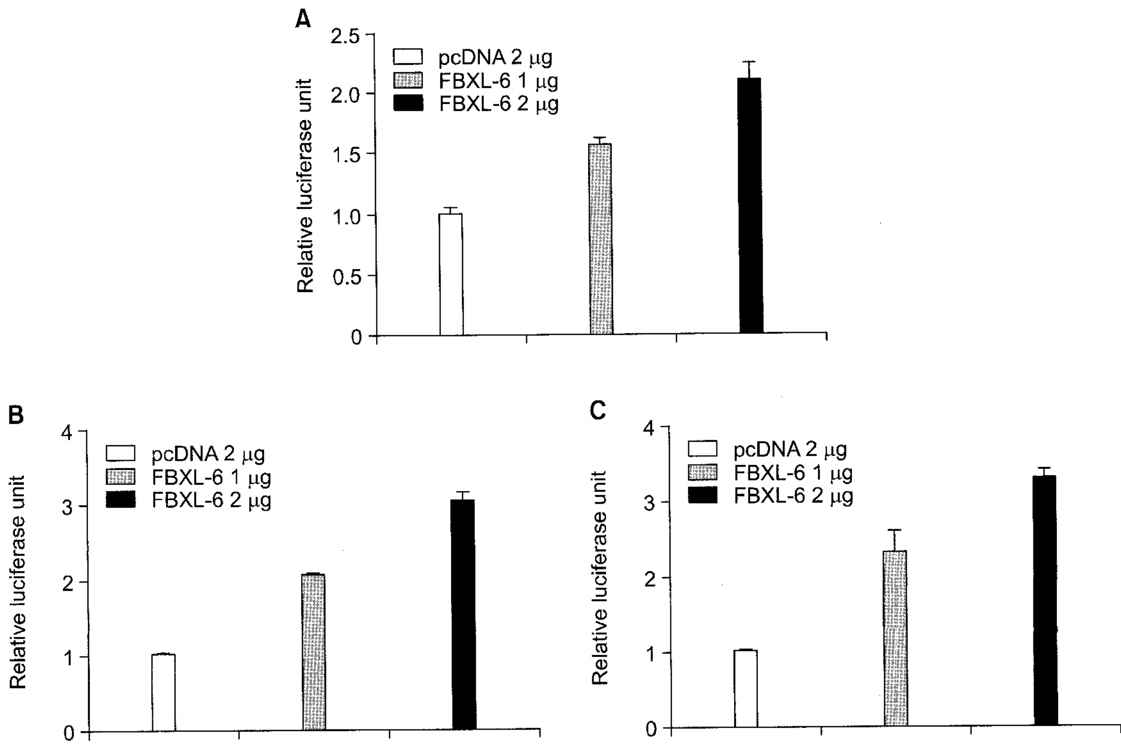


**Fig. 3.** The effect of full-length FBXL-6 on the E2F-mediated transcription in various cancer cells. Each transfection mixture consisted of 1 μg of $(E2F)_6$/Min-Luc reporter vector and 0.2 μg of CMV-LacZ internal control. 1 or 2 μg of full-length cDNA expression vector for FBXL-6 gene or 2 μg of pcDNA3.0 (control) were co-transfected into HCT116 cells (A), SNU-354 hepatocellular carcinoma cells (B) or SNU-484 gastric cancer cells (C). Luciferase activities in the cell lysate were measured and finally normalized for the transfection efficiency.

Some of these proteins are responsible for the recognition of G1 cell cycle regulators such as cyclin E,[25)] p27 cdk inhibitor[26)] and p130,[27)] a member of pRB family. The FBXL-6, one kind of F-box proteins, has recently been cloned by two groups[19,20)] but its functions in the cell are yet to be elucidated. In this study, we hypothesized that like the previously reported F-box proteins the FBXL-6 may also modulate the function of certain cell cycle regulators, and, as the initial step to resolving this hypothesis, we estimated the effect of this molecule on the transcriptional activity of E2Fs, key transcription factors for cell cycle control. Both 5'-truncated and full-length gene of FBXL-6 could increase the E2F-mediated transcriptional activity in various kinds of cells including HCT116 CRC cells. In addition, the mRNA of this gene was shown expressed in a diverse pattern in different cell types. Our results and the known functions of typical F-box proteins strongly suggest the possibility that FBXL-6 may regulate the activity or stability of a protein constituting the E2F pathway via a post-translational modification. Further studies would be required for clarifying the mechanisms of increased E2F-mediated transcriptional activity by FBXL-6 and its biological significance.

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## REFERENCES


1) Black AR, Azizkhan-Clifford J. Regulation of E2F: a family of transcription factors involved in proliferation control. *Gene* 1999; 237: 281-302.
2) Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. *Nat Rev Mol Cell Biol* 2002; 3: 11-20.
3) Mundle SD, Saberwal G. Evolving intricacies and implications of E2F1 regulation. *FASEB J* 2003; 17: 569-574.
4) Helin K. Regulation of cell proliferation by the E2F transcription factors. *Curr Opin Genet Dev* 1998; 8: 28-35.
5) Stevens C, La Thangue NB. E2F and cell cycle control: a double-edged sword. *Arch Biochem Biophys* 2003; 412: 157-169.
6) Yamasaki L. Growth regulation by the E2F and DP transcription factor families. *Results Probl Cell Differ* 1998; 22: 199-227.
7) DeGregori J, Kowalik T, Nevins JR. Cellular targets for activation by the E2F1 transcription factor include DNA synthesis- and G1/S-regulatory genes. *Mol Cell Biol* 1995; 15: 4215-4224.
8) Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP. A human DNA segment with properties of the gene that predisposes toretinoblastoma and osteosarcoma. *Nature* 1986; 323: 643-646.
9) Morris EJ, Dyson NJ. Retinoblastoma protein partners. *Adv Cancer Res* 2001; 82: 1-54.
10) Livingston DM, Kaelin W, Chittenden T, Qin X. Structural and functional contributions to the G1 blocking action of the retinoblastoma protein. (the 1992 Gordon Hamilton Fairley Memorial Lecture). *Br J Cancer* 1993; 68: 264-268.
11) Mulligan G, Jacks T. The retinoblastoma gene family: cousins with overlapping interests. *Trends Genet* 1998; 14: 223-229.
12) Stevaux O, Dyson NJ. A revised picture of the E2F transcriptional network and RB function. *Curr Opin Cell Biol* 2002; 14: 684-691.
13) Nevins JR. Cell cycle targets of the DNA tumor viruses. *Curr Opin Genet Dev* 1994; 4: 130-134.
14) Nevins JR. The Rb/E2F pathway and cancer. *Hum Mol Genet* 2001; 10: 699-703.
15) Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* 1997; 91: 209-219.
16) Feldman RM, Correll CC, Kaplan KB, Deshaies RJ. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* 1997; 91: 221-230.
17) Patton EE, Willems AR, Tyers M. Combinatorial control in ubiquitin-dependent proteolysis: don't Skp the F-box hypothesis. *Trends Genet* 1998; 14: 236-243.
18) Kipreos ET, Pagano M. The F-box protein family. *Genome Biol* 2000; 1: REVIEWS3002.1-3002.7
19) Winston JT, Koepp DM, Zhu C, Elledge SJ, Harper JW. A family of mammalian F-box proteins. *Curr Biol* 1999; 9: 1180-1182.
20) Cenciarelli C, Chiaur DS, Guardavaccaro D, Parks W, Vidal M, Pagano M. Identification of a family of human F-box proteins. *Curr Biol* 1999; 9: 1177-1179.
21) Yanagisawa K, Kondo M, Osada H, Uchida K, Takagi K, Masuda A, Takahashi T, Takahashi T. Molecular analysis of the FHIT gene at 3p14.2 in lung cancer cell lines. *Cancer Res* 1996; 56: 5579-5582.
22) Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998; 67: 425-479.
23) Sakamoto KM. Ubiquitin-dependent proteolysis: its role in human diseases and the design of therapeutic strategies. *Mol Genet Metab* 2002; 77: 44-56.
24) Nalepa G, Wade Harper J. Therapeutic anti-cancer targets upstream of the proteasome. *Cancer Treat Rev* 2003; 29: 49-57.
25) Strohmaier H, Spruck CH, Kaiser P, Won KA, Sangfelt O, Reed SI. Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. *Nature* 2001; 413: 316-322.
26) Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol* 1999; 1: 193-199.
27) Tedesco D, Lukas J, Reed SI. The pRb-related protein p130 is regulated by phosphorylation-dependent proteolysis via the protein-ubiquitin ligase SCF (Skp2). *Genes Dev* 2002; 16: 2946-2957.

# HCT116 세포에서 FBXL-6 유전자에 의한 E2F 전사 활성 조절

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F-box protein은 Skp1p-Cullin-F-box protein (SCF) complex의 구성 요소로써 ubiquitin-dependent proteolysis 과정 중 분해될 기질의 인식작용에 중추적 역할을 수행한다. 일부 F-box protein들은 cyclin E, p27 등과 같은 주요 cell cycle 조절자의 분해에 관련되어 있다고 알려져 있다. 본 연구에서는 유전자 동정에 대한 보고만 있을 뿐 상위 혹은 하위 유전자와의 구체적인 관계 및 그 작용기전에 대한 연구가 전무한 F-box and leucine-rich repeat protein 6 (FBXL-6)가 세포 성장 조절의 중추적 전사인자인 E2F의 활성 조절에 미치는 영향에 대하여 조사하였다. 5' 말단 부위의 일부 염기서열이 결여된 FBXL-6 유전자를 내재적 E2F 전사 활성이 억제되어 있는 대장암 세포주 HCT116에서 과발현시킨 경우 E2F의 활성이 유의하게 증가됨을 확인하였다. RT-PCR 분석에 의하면 FBXL-6의 mRNA는 거의 모든 암세포에서 발현됨을 관찰하였다. 또한 full-length 발현 벡터를 사용하여 HCT116 대장암 세포와 간암 및 위암 세포에서 E2F 전사 활성에 대한 FBXL-6 유전자의 과발현에 따르는 효과를 조사한 결과, 대부분의 암세포에서 E2F 전사활성이 증가됨을 거듭 확인하였다. 이상의 결과로 볼 때 FBXL-6 유전자는 대부분의 세포에서 발현되며, 과발현 시 세포 내에서의 E2F 전사 활성을 증가시킬 수 있음이 확인되었다. 이것은 FBXL-6 유전자가 E2F pathway상의 어떤 단백질에 대한 post-translational modification을 통해 그 활성이나 안정성을 조절할 가능성이 있음을 시사하는 결과라 할 수 있다.

**Key Words:** E2F 전사인자, F-box 단백질, FBXL-6, HCT116 세포주