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c-myc suppressor FBP-interacting repressor for cancer diagnosis and therapy

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1. ABSTRACT

Based on the genetic background of cancer, we have been trying to develop novel diagnostic and therapeutic strategies against human cancers. *c-myc* gene activation has been detected in many human cancers, indicating a key role of *c-myc* in tumor development. Thus targeting *c-myc* gene suppression is a promising strategy for cancer treatment. Recently, an interaction between FIR (FUSE-Binding Protein-Interacting Repressor) and TFIIH/p89/XPB helicase was found to repress *c-myc* transcription and so might be important for suppressing tumor formation. Previously, we have shown that the expression of splicing variant of FIR is elevated in colorectal cancer tissues and promotes tumor development by disabling FIR-repression to sustain high levels of c-Myc, opposing apoptosis in cancer cells. In this study, FIR recombinant adenovirus vector induces tumor growth suppression against tumor xenografts in animal model experiment. Together, one clue to the development of cancer diagnosis and therapies directed against c-Myc may go through FIR and its splicing variant.

2. INTRODUCTION

Surgical excision alone infrequently results in long-term survival for advanced gastrointestinal cancers, thus efforts are now focused on combined treatments in an attempt to improve local control and eliminate micro-metastasis at the time of operation (1, 2). Despite improvement of surgical treatment and chemotherapies to advanced cancers, the prognosis is poor in those tumors. Significant growth suppression was observed by infection with p53 recombinant adenoviral vector (Ad-p53) in human esophageal squamous cell carcinoma cell lines. Our group has performed a clinical trial of Ad-p53 for un-resectable advanced human esophageal cancer treatment. So far the efficiency of Ad-p53 for cancer treatment is not satisfactory, thus a novel molecular target is required.

c-Myc plays an essential role in cell proliferation and tumorigenesis. *c-myc* activation was also shown to be required for skin epidermal and pancreatic beta-cell tumor maintenance in c-MYC-ER^{TAM} transgenic mice (3). *c-myc* expression level reflects interferon-gamma production in

colorectal cancer tissues and this host's immune response was associated with the better long-term survival of colorectal cancer patients (4). These observations have encouraged us for the future development of cancer therapies targeting *c-myc*. The Far UpStream Element (FUSE) is a sequence required for proper expression of the human *c-myc* gene. The FUSE is located 1.5 kb upstream of *c-myc* promoter P1, and binds the FUSE Binding Protein (FBP), a transcription factor stimulating *c-myc* expression in a FUSE dependent manner (5, 6). Yeast two-hybrid analysis revealed that FBP binds to a protein that has transcriptional inhibitory activity termed the FBP Interacting Repressor (FIR), and FIR was found to engage the TFIIH/p89/XPB helicase and repress *c-myc* transcription by delaying promoter escape (7).

Up to 60% of all human genes present at least one alternative splice variant (8). Alternative splicing has been documented to play a significant role in human disease including cancer (9). We have previously reported that a splice variant of the *c-myc* repressor FIR plays an important role in the pathogenesis of human colorectal cancer (10). A splicing variant of FIR that lacks exon2 (FIRdel/exon2), expresses mainly in tumor tissues, but not in the adjacent normal tissue. FIRdel/exon2 failed to repress c-Myc and inhibited FIR-induced apoptosis suggesting an important role for this splicing variant of FIR in the tumorigenesis of human colorectal cancer (10). In this study, enforced FIR expression by adenovirus vector in tumors for cancer therapy and detection of FIR and/or FIRdel/exon2 in the peripheral blood for cancer diagnosis are discussed.

3. MATERIALS AND METHODS

3.1. Plasmids

Full-length FIR cDNA (HA-FIR) and the FIR deleted of its first seventy-seven amino acids (HA-FIRdel/N77), and the alternatively spliced form of FIR lacking exon 2 (HA-FIRdel/exon2), obtained from cancer tissues, were cloned into the pCGNM2 vector plasmid (10), respectively, to introduce the hemagglutinin (HA)-tag at their amino termini. The human c-Myc expression vector, pcDNA3.1-*c-myc*, GeneStorm® Expression-Ready Clones (Invitrogen Co., AL) was purchased and c-Myc expression was confirmed by western blot using anti-c-Myc (Upstate Biotechnology, NY). Full-length FIR cDNA was cloned into pcDNA3.1 to generate pcDNA3.1-FIR.

3.2. Human tissue samples

Tissues from 15 cases of primary colorectal cancer were surgically excised. Written informed consent was obtained from each patient prior to surgery. The tumor samples were obtained from tumor epithelium immediately after operative excision tissues and the corresponding non-tumor epithelial samples were 5-10 cm from the tumor. All excised tissues were immediately placed into liquid nitrogen and stored at –80˚C until analysis.

3.3. Immunocytochemistry

HeLa cells were grown on coverslips overnight and then transfected with plasmids using Lipofectamine Plus reagents (Invitrogen, Carlsbad, CA). At the indicated time after plasmid transfection, cells were treated for immunocytochemistry as previously described (10). The primary antibodies, mouse monoclonal anti-HA (Santa Cruz Biotechnology, CA), rabbit polyclonal anti-c-Myc (Upstate Biotechnology, NY), were diluted 1:500 and 1:200 in the blocking buffer, respectively. The primary rabbit polyclonal antibody against FIR was prepared using two synthetic peptides GDKWKPPQGTDSIKME and EVYDQERFDNSDLSA simultaneously immunized to enhance the possibility of antibody production (JAPAN BIO SERVICES Co Ltd, Saitama, Japan), and was diluted 1:200 in the blocking buffer. The coverslips were incubated at room temperature for 1 hr. After washing with PBS, the secondary antibodies, Alexa Fluor™ 488-conjugated goat anti-rabbit or 594-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes, Eugene, OR) was used at 1:1,000 dilution. DNA was counterstained with DAPI III (Vysis, Abbott Park, IL) and cells were observed under immunofluorescence microscopy (Leica QFISH; Leica Microsystems, Tokyo, Japan).

3.4. Apoptosis detection

Apoptotic cells were detected by TUNEL assay according to the manufacturer's instructions (Apoptosis Detection System, Fluorescein. Promega, WI, USA). Briefly, HeLa cells cultured on cover glasses were fixed with paraformaldehyde at 4˚C for 10 min on ice and permeabilized with 0.5% Triton-X-100 solution in PBS for 5 min. After washing with PBS twice, apoptotic cells were visualized through detection of inter-nucleosomal fragmentation of DNA using *in situ* nick-end labeling with terminal deoxynucleotidyl transferase (TdT) and FITC-labeled dUTP (MEBSTAIN Apoptosis Kit: Medical & Biological Laboratories, Japan). DNA was counterstained either with DAPI III for microscopy.

3.5. Protein extraction and immunoblotting

Frozen tissue samples were dissolved in lysis buffer (7M urea, 2M thiourea, 2% 3-[(3-Cholamidopropyl) dimethylammonio-] 1-propanesulfate (CHAPS), 0.1 M Dithiothreitol (DTT), 2% IPG buffer (Amersham Pharmacia Biotech, Buckinghampshire, UK), 40 mM Tris) using a Polytron homogenizer (Kinematica, Switzerland) following centrifugation (100,000 x g) for 1hr at 4˚C. The amount of protein in the supernatant was measured by protein assay (Bio-Rad, Hercules, CA). The proteins were separated by electrophoresis on 8 % polyacrylamide gels and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA) in a tank transfer apparatus (Bio-Rad, Hercules, CA). The membrane was blocked with 5% skim milk in PBS for 1hr. Rabbit polyclonal anti-FIR antibody and goat polyclonal anti-beta-actin antibody (Santa Cruz, Santa Cruz, CA) diluted 1:1,000 and 1:500, respectively, in blocking buffer were used as primary antibodies. Goat anti-rabbit IgG horseradish peroxidase conjugate (HRP) (Jackson, West Grove, PA) diluted 1:3,000 and rabbit anti-goat IgG HRP (Cappel, West Chester, PA) diluted 1:500 were used as secondary antibodies. Antigens on the membrane were detected by ECL™ detection reagents (Amersham Pharmacia Biotech). The intensity of each band was measured by NIH Image.

3.6. Reverse transcriptase (RT)-PCR and real-time quantitative PCR

Total RNA and genomic DNA were extracted from tumor and non-tumor epithelial tissues with the RNeasy™ Mini Kit and the DNeasy™ Tissues Kit (Qiagen). cDNA was synthesized from total RNA with the 1st strand cDNA Synthesis Kit for RT-PCR (Roche, Mannheim, Germany). Using the cDNA as a template, FIR cDNA was amplified with suitable primers by RT-PCR: forward 5'-GGCCCCATCAAGAGCATC -3', reverse 5'-GGGGCTGGGCCAGGGTCAG -3'. For control, GAPDH cDNA was amplified. The amino terminal region of FIR was amplified by RT-PCR with primers: forward 5'-AGACAGCGGAAGGAGCAAGAGTGG-3', reverse 5'-CTGTGCAGCTTCGGGGACCTCATA -3'. The PCR product was loaded on a 2.5 % agarose gel (Promega, WI), purified by Gel Extraction Kit™ (Qiagen) and cloned with the pGEM®-T Easy vector system (Promega, WI) for DNA sequencing.

3.7. MTT assay

The effects of FIR on cell survival were examined by the MTT method of Mosmann (11) as described previously (12). HeLa and esophageal squamous cell carcinoma cells (T.Tn) were infected with Ad-FIR or control Ad-LacZ at MOI 0.1 to 10, and cultured for 3 days. Cell viability was quantified by measuring the absorbance at 570 nm after incubation with MTT for 4 hr. The results are shown as percentages of the control results.

3.8. FIR adenovirus vector

The recombinant adenoviral vectors that express FIR was constructed through homologous recombination in Escherichia coli using the AdEasy XL system (STRATAGENE, Cedar Creek, Texas). *Hind*III-*Pme*I fragment of pcDNA3.1-FIR or *Hind*III-*Eco*RV fragment of pcDNA3.1-CMV-LacZ were cloned into the *Hind*III–*Eco*RV sites of pShuttle-CMV, generating pShuttle-CMV-FIR or pShuttle-CMV-LacZ, respectively. The resultant shuttle vectors were linearized with *Pme*I digestion and subsequently cotransfected into *Escherichia coli* BJ5183-AD-1. The recombinants were linearized with *Pac*I digestion and transfected into the E1 trans-complementing 293 cell line to generate Ad-FIR and Ad-LacZ. The viruses were propagated in the adenovirus packaging 293 cell line, and purified by double CsCl density gradient centrifugation, followed by dialysis against 10 mM Tris buffer (pH 8.0) with 10% glycerol. The viruses were aliquoted and stored at −80°C until usage. The virus titer was determined by conventional limiting dilution on 293 cells.

3.9. Tumor xenografts in animal model experiments

The *in vivo* inhibition of tumorigenicity of esophageal squamous cell carcinoma cells (TE-2) was examined by Ad-FIR or Ad-LacZ injection. $5x10^6$ cells of TE-2 inoculated with Ad-FIR or Ad-LacZ at an MOI of 10, were injected beneath the skin of right thigh of nude mice (balbc/nu/nu, 6-week birth, male). Tumor growth was observed and measured the long and short diameter for tumor volume calculation. Thirty days after inoculation, tumor grew up to 5-8 mm in diameter in 9 of 9 mice (100%).

4. RESULTS

4.1. The amino terminal domain of FIR is required to represses endogenous c-Myc expression.

Previous studies revealed that the amino terminus of FIR was necessary to repress transcription from the *c-myc* promoter of a transfected reporter plasmid (10). To test the effect of FIR on the endogenous c-Myc expression, HA-tagged, full length FIR (HA-FIR) was expressed in HeLa cells, the transfected cells were identified using anti-HA and c-Myc expression in transfected cells was visualized by immunostaining with anti-c-Myc (Figure 1A upper panels). c-Myc levels were greatly diminished in HA-FIR expressing cells (arrowheads), but were unperturbed in HA-negative cells, demonstrating that FIR represses endogenous c-Myc expression through a cell autonomous mechanism. To test if the amino-terminal region of FIR is required for the suppression of c-Myc, its amino terminal deletion mutant (HA-FIRdel/N77) was transfected into HeLa cells (Figure 1A lower panels). In contrast to the full-length protein, deleting its amino-terminus enfeebled FIR's repressor activity (arrows).

4.2. FIR-induced apoptosis is prevented by enforced expression of c-Myc.

Because FIR depresses native *c-myc* expression, we tested the influence of enforced FIR-expression upon apoptosis. HA-FIR and HA-FIRdel/N77 were transfected into HeLa cells and apoptosis was examined by TUNEL assay (Figure 1B). HA-FIR induced apoptosis with DNA fragmentation (Figure 1B upper panel with arrows), whereas little apoptosis occurred in cells transfected with HA-FIRdel/N77 or the control vector. Equivalent expression of HA-FIR and HA-FIRdel/N77 protein in HeLa cells was confirmed by immunoblot (data not shown).

If FIR suppression of *c-myc* drives apoptosis, then bypassing this repression should rescue cells from death. Expression from an exogenous promoter enabled the elevation of c-Myc levels even when co-transfected with FIR (Figure 1C right upper panel; arrowheads). Augmented c-Myc expression protected HeLa cells from the FIR-driven apoptosis. The extent of apoptosis driven by FIR declined from 21.1% to 4.2% when c-Myc expression was enforced (Figure 1D). The elevating c-Myc levels also prevented FIR-induced nuclear swelling and degradation (Figure 1C right lower panels). These results indicate that increasing FIR levels, triggers apoptosis, most likely due to *c-myc* suppression.

4.3. FIR is paradoxically upregulated in colorectal cancer correlating with increased c-Myc.

It is well known that c-Myc is overexpressed in the majority of colorectal cancers due to deregulation of *c-myc* expression. Thus we hypothesized that cancer cells must escape FIR-repression of *c-myc*. FIR itself might be downregulated in cancer, or alternatively the effector actions of FIR in transcription and apoptosis might be disabled. To test if FIR is downregulated in colorectal cancer, FIR protein levels in tumors and normal tissue from 10 cases were examined by immunoblot. Unexpectedly,

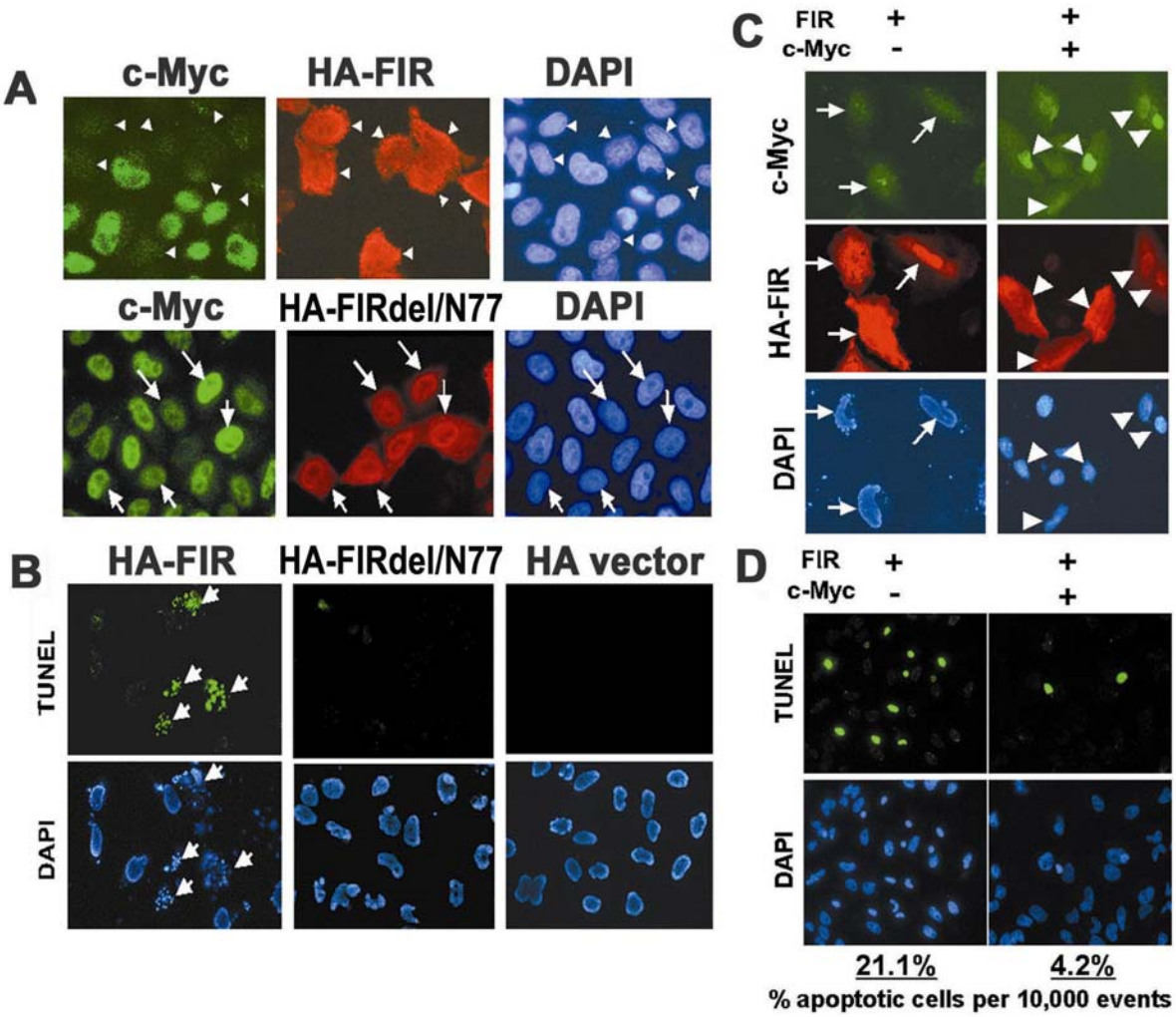

Figure 1. FIR suppresses c-Myc and induces apoptosis; the amino terminus is necessary for the activities. (A) 100 fmol of HA-FIR or HA-FIRdel/N77 was transfected into HeLa cells in 6-well plate. After 24 hours, cells were immunostained with antibodies against c-Myc (left, green) or HA (middle, red). Arrowheads and arrows show the cells in which HA-FIR and HA-FIRdel/N77 were expressed, respectively. c-Myc expression was markedly reduced in most HA-FIR-expressing cells (arrowheads) when compared to HA-FIRdel/N77-expressing cells (arrows). (B) Examination of apoptotic cells by TUNEL assay. 150 fmoles of HA-FIR, HA-FIRdel/N77, vacant vector plasmids were transfected to HeLa cells in 6-well plate and 48 hours later TUNEL assay was performed. Upper panels show apoptotic cells (arrows) after HA-FIR transfection to HeLa cells. Middle and right panels are HA-FIRdel/N77 and control (HA vacant vector) transfected cells, respectively. (C) 600ng of pcDNA3.1-FIR were transfected into semiconfluent HeLa cells on a 6-well plate with or without 60 ng of c-Myc expression plasmids (pcDNA3.1-*c-myc*). c-Myc expression was remarkably suppressed when FIR alone was transfected (left, top, arrows) whereas overall c-Myc expression was elevated when *c-myc* plasmids were cotransfected with FIR plasmids (right, top, arrowheads). Staining with DAPI showed that nuclei were swollen and degraded in FIR transfected cells (left, bottom, arrows), vs. normal appearing in FIR and c-Myc co-expressing cells (right, bottom, arrowheads). (D) The number of apoptotic cells caused by FIR was drastically reduced when co-expressed with c-Myc. The percentage of apoptotic cells caused by FIR alone was 21.1%, but decreased to 4.2 % when FIR and c-Myc were co-expressed.

FIR levels were actually increased in most colorectal cancer tissue compared with the corresponding non-tumor epithelium (Figure 2A). To determine if the increased FIR levels resulted from increased levels of FIR mRNA, RNA from colorectal cancer cells and normal colonic epithelium were examined by RT-PCR (Figure 2B). All cases, except Case 2, showed higher FIR mRNA levels in tumors compared with non-tumor tissue. FIR mRNA and protein levels paralleled each other in all cases.

4.4. An alternatively spliced form of FIR is expressed in tumors, but not the adjacent normal tissue

Either FIR must be defective, or the *c-myc* promoter must somehow be made resistant to FIR. RT-PCR of full-length FIR cDNAs isolated from HeLa cells or colorectal cancer tissues using primers to amplify the amino terminal

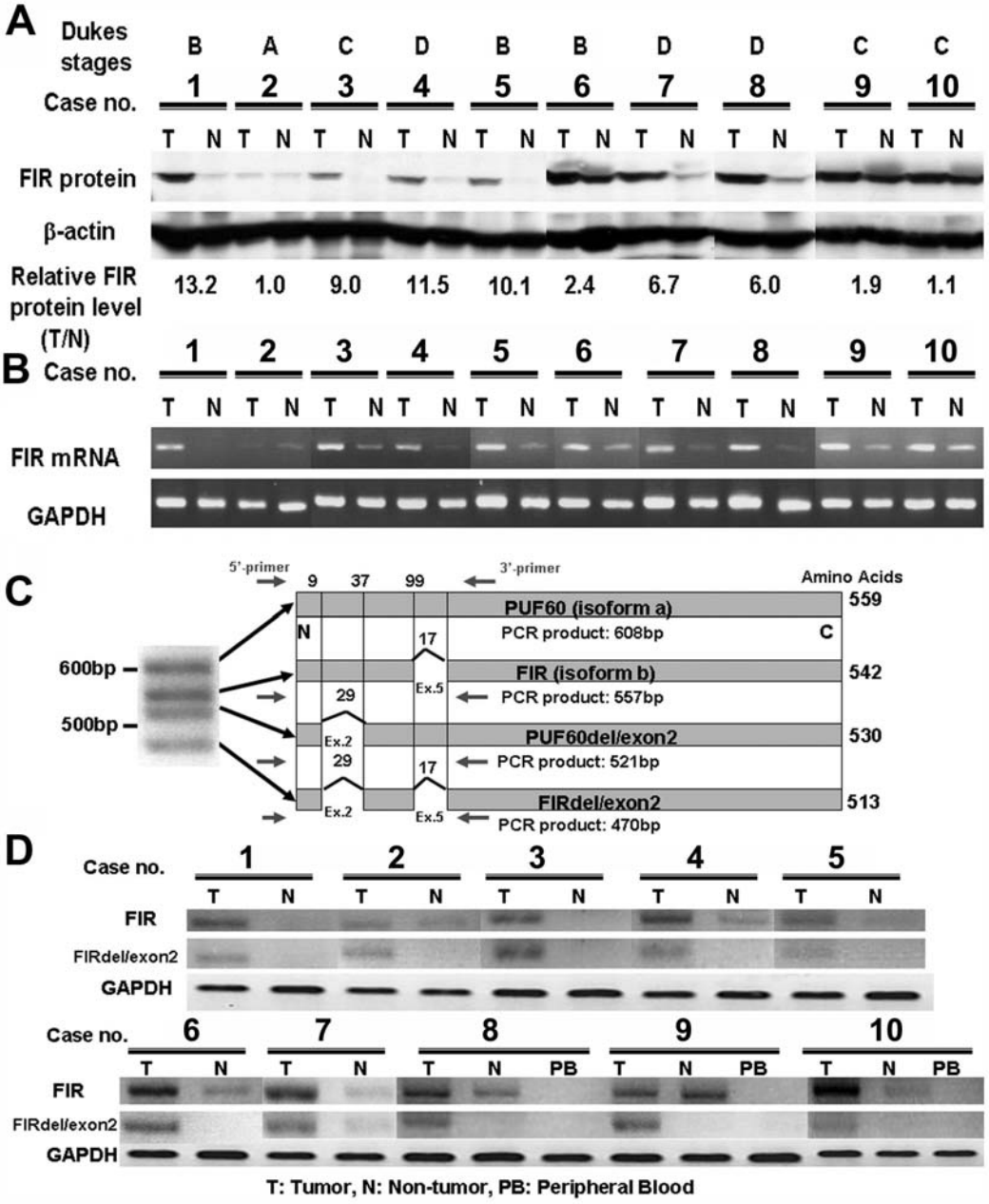

Figure 2. FIR was paradoxically overexpressed in colorectal cancer tissues. (A) Total protein lysates were prepared from matched samples of tumor (T) and adjacent non-tumor epithelial tissue (N). Equal amounts of protein from each pair were resolved on 8% polyacrylamide gel and immunoblotted with anti-FIR antibody. Immunoblotting was also performed with β-actin antibody as a loading control. Intensity of each band was measured by NIH Image and the relative mean of FIR protein levels between (T) and (N) with β-actin were calculated at the bottom of the figure. FIR expression significantly increased in (T) compared with corresponding (N). (B) Total RNAs were prepared from matched samples of (T) and (N) and RT-PCR was performed. FIR mRNA levels were comparative with the protein levels of each case. GAPDH mRNA levels are also showed as internal control. (C) Alternative spliced form of FIR that lacks exon 2 (FIRdel/exon2) is expressed specifically in human colorectal cancer tissues. FIR cDNA, obtained from HeLa cells or colorectal cancer tissues, was amplified by PCR using primers for the amino terminal region of FIR. Four distinct bands were observed in 2.5% agarose gel. Each band was excised and DNA was eluted, then sequenced. The PCR products were four alternative spliced forms of FIR that lack exon 2 and/or exon 5, whose sizes were 608bp, 557bp, 521bp, and 470bp, respectively. Exon 2 and exon 5 consists of twenty-nine and seventeen amino acids. Exon 2 locates at amino terminal suppression domain. (D) RT-PCR using cDNA obtained from colorectal cancer tissues was performed as in (A). Although both FIR and FIRdel/exon2 expressions increased in (T) compared with (N), FIRdel/exon2 was only expressed in tumor of all cases.

region revealed the four variants (Figure 2C; FIR and PUF60, FIRdel/exon2 and PUF60del/exon2) expected from the alternative utilization of the two optional exons. DNA sequencing confirmed that the four products reflected all combinations of inclusion/exclusion of exons 2 and 5 (Figure 2C). To examine if the alternative splicing of FIR is a tumor specific event, FIR cDNA isolated from tumors and normal tissues of several cases were examined by RT-PCR. The splicing variant FIRdel/exon2 was only observed in tumor tissues and not in adjacent normal tissues or in blood cells from most of the matched cases (Figure 2D).

4.5. FIR adenovirus indicates antitumor effect on human cancer cells *in vitro* and *in vivo*

Based on the concept of genetic alteration in carcinogenesis, gene therapy has increasingly developed as an alternative to conventional cancer therapy. We have previously reported that FIR induces apoptosis thus applicable for cancer gene therapy (10). Clinically, the recombinant adenoviral vector is expected to be a good agent for introducing genes of interest into cancer cells due to its high transduction efficiency. In this study, preclinical studies of adenoviral-mediated FIR gene delivery are required to confirm the anti-cancer effect *in vivo* and *in vitro*.

The effects of FIR on the survival of cervical squamous cell carcinoma (HeLa), and esophageal cancer cells (T.Tn) were examined by infection with Ad-FIR followed by MTT assay. The viability of Ad-FIR-infected cells were much lower than that of control Ad-LacZ-infected cells at each MOI ranging from 0.1 to 10. This difference may be attributed to the expression of FIR. Almost complete loss of surviving cells by infection with Ad-FIR at an MOI of 10 suggests the cytocidal effects of FIR. The suppression of the viability was more prominent to HeLa cells than to T.Tn cells, possibly reflecting the protein expression levels of FIR. The growth of TE-2 cells injected into nude mice was also suppressed by infection with Ad-FIR. Significant growth retardation was observed with Ad-FIR but not with control Ad-LacZ (data not shown).

In the above set of experiments, we demonstrated that adenovirus-mediated FIR gene transduction efficiently suppressed tumor growth of human cancer cell lines both *in vitro* and *in vivo*. Precise determination of the mechanism underlying the growth suppressive effect by FIR will require further investigation.

5. DISCUSSION

In this study, FIR adenovirus vector significantly inhibited tumor growth in mice animal model. The mechanism of anti-tumor effect by overexpression of FIR recombinant adenovirus vector (Ad-FIR) appears to be the induction of apoptosis. We herein report the findings of a preclinical study that reveal the efficacy of Ad-FIR treatment on human cancers both *in vitro* and *in vivo*. Tumor cells inoculated with Ad-FIR demonstrated significant growth retardation at an MOI of 10. This strategy could be useful for patients to whom relatively curative resection has been performed for locally advanced cancers and possible micro residual cancers are present.

This study demonstrated that FIR strongly repressed endogenous *c-myc* transcription and induced apoptosis. Most importantly, a splicing variant of FIR, FIRdel/exon2, found frequently in human primary colorectal cancer tissues, not only lacked the c-Myc-suppressing and apoptosis-inducing action of FIR, but prevented normal FIR from performing these activities. Thus FIRdel/exon2 may contribute to tumor progression by enabling higher levels of *c-myc* expression and greater resistance to apoptosis in tumors than in normal cell (Figure 3A). Though splice variants can function as dominant negative inhibitors and interfere with the wild type, their selective occurrence in tumors has not been proven. To demonstrate how the alternative splicing of FIR is differentially regulated between tumors and the surrounding normal tissue promises to expose further links between the earliest events in carcinogenesis with tumor progression. In addition FIR and/or FIRdel/exon2 are elevated in colorectal cancer tissues, circulating FIR and/or FIRdel/exon2 proteins or mRNAs in peripheral blood are potent biomarker for cancer detection. The value of FIR and/or FIRdel/exon2 detection for cancer diagnosis is under investigation.

Our experiments indicate that the FIR is an important player in these processes by directly regulating *c-myc*. Evidence for this scheme includes: 1) the amino terminus of FIR, essential for *c-myc* suppression, is also necessary for induction of apoptosis. 2) a splicing variant of FIR lacking exon 2 in the amino terminus failed not only to suppress c-Myc expression but to induce apoptosis. 3) enforced expression of c-Myc rescued cells from FIR-induced apoptosis. Together, FIRdel/exon2 protein transport inhibition into the nucleus and/or FIRdel/exon2 mRNA export inhibition into the cytoplasm may be promising molecular targets for future cancer therapy (Figure 3B).

Recently, Puf60, another FIR splicing variant having exon 5, directly binds to splicing factor SF3b with $U2AF^{65}$ (13, 14) and inhibition of SF3b by natural chemicals demonstrated strong antitumor effect (15, 16). Given the central role of c-Myc in the development of many cancers and inhibition of splicing function of FIR homologue (or FIR itself) with SF3b indicates strong antitumor activity, one route to the development of cancer therapies directed against c-Myc and splicing of SF3b inhibition may go through FIR and its variants (Figure 3B). Further studies are required in this field.

According to recent cancer gene therapy reports, adenovirus-mediated p53 gene transfer is frequently used, together with cis-dichloro-diammineplatinum (CDDP) administration or ionizing radiation (17). As for Ad-FIR vector, the transduction efficiency was relatively low, but an MOI of 10 was sufficient to show the efficacy in preclinical trials. Because treatment

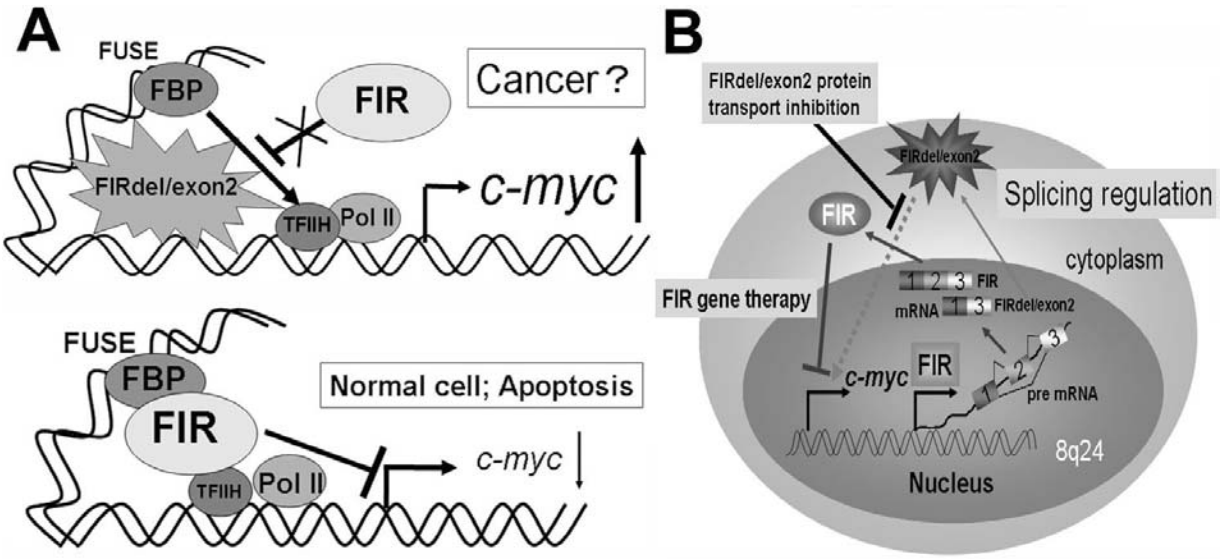

Figure 3. (A) c-Myc plays a critical role in cell proliferation and tumorigenesis. The Far UpStream Element (FUSE) is a sequence required for proper expression of the human *c-myc* gene. The FUSE is located 1.5 kb upstream of *c-myc* promoter P1, and binds the FUSE Binding Protein (FBP), a transcription factor stimulating *c-myc* expression in a FUSE dependent manner. FIR interacts with the central DNA binding domain of FBP. FIR was found to engage the TFIIH/p89/XPB helicase of TFIIH and repress *c-myc* transcription by delaying promoter escape. In this study, we show that a splice variant of the *c-myc* repressor FIR plays an important role in the pathogenesis of human colorectal cancer. A FIRdel/exon2, existing only in tumors, but not in the adjacent normal tissue, failed to repress c-Myc and inhibited FIR-induced apoptosis suggesting an important role for this splicing variant of FIR in the tumorigenesis of human colorectal cancer. (B) FIR gene therapy is a promising tool for cancer treatment. FIRdel/exon2 protein transport inhibition into the nucleus and/or FIRdel/exon2 mRNA export inhibition into the cytoplasm may be potential molecular targets for future cancer therapy leading *c-myc* suppression. FIR and/or FIRdel/exon2 mRNAs in the peripheral blood are potent biomarkers for cancer detection.

response is strongly associated with survival, combination treatment with standard chemoradiation and Ad-FIR may be an attractive modality in the future. FIR and its splicing variants are fascinating targets for cancer diagnosis and treatment.

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Abbreviations: FUSE: Far Upstream Element; FBP: FUSE-Binding protein; FIR: FBP Interacting Repressor, Ad: adenovirus

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