Acta Biochim Biophys Sin (2009): 456–463 | © The Author 2009. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmp032.
Advance Access Publication 29 April 2009

Sequences near both termini of the C/EBPβ mRNA 3′ untranslated region are important for its tumor suppression activity

Haizhen Wang, Ying Wang, Li Sun, and Dinggan Liu*

State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
*Correspondence address. Tel: +86-21-54921135; Fax: +86-21-54921011; E-mail: dgliu@sibs.ac.cn; kinverskur@sohu.com

The 3′ untranslated region (3′ UTR) of eukaryotic mRNA is an important regulation element that affects not only mRNA translation, but also cell growth. We had found that the 3′ UTR of CCAAT-enhancer-binding protein β (C/EBPβ) mRNA had tumor suppression activity. Herein, we reported that deletion of two short sequences at both termini of the C/EBPβ 3′ UTR reduced the tumor suppression activity of this 3′ UTR, as demonstrated by reduced cell growth, colony formation ability, and tumorigenicity in nude mice. It is noteworthy that the only deletion of a single such sequence was enough for the reduction of tumor suppression effect, and the reducing effect of deletion of the sequence near 3′ terminus was stronger. Therefore, specific short sequences in the C/EBPβ 3′ UTR are crucial for the tumor suppression activity of C/EBPβ.

Keywords C/EBPβ 3′ UTR; tumor suppression; deletion

Received: January 7, 2009 Accepted: February 27, 2009

Introduction

Since the last decade, several eukaryotic mRNA 3′ untranslated regions (3′ UTRs) with tumor suppression function were found. Up to date, six 3′ UTRs, from the mRNAs of α-tropomyosin [1], prohibitin [2], ribonucleotide reductase R1 and R2 [3], mel-18 [4], and CCAAT-enhancer binding protein beta (C/EBPβ), a transcription factor also known as nuclear factor of interleukin 6 (NF-IL6) [5], have been identified as tumor suppressors.

As 3′ UTR does not encode proteins, its role in gene expression regulation is supposed to be due to its interactions with cellular protein factors [1]. However, the molecular mechanisms of tumor suppression by most 3′ UTRs remain unclear.

In our previous research, we showed that the 0.28 kb fragment, i.e. the middle part, of C/EBPβ 3′ UTR, had strong tumor suppression effect in SMMC-7721 hepatoma cells; and, by stable transfection of that cell line with plasmid p14-6 (see Materials and Methods section), we had obtained a lot of revertants that highly expressed the RNA from C/EBPβ 3′ UTR, including Cl1. cDNA array analysis for Cl1 cells revealed that several genes related to suppression of malignant phenotype, like p53-related genes, and some cytoskeletal protein genes, were up-regulated, while some genes that promote malignancy were down-regulated [6]. These results suggested that this part of C/EBPβ 3′ UTR might regulate genes related to cellular phenotype to display its tumor suppressor function. We also found that the C/EBPβ 3′ UTR interacts with several cellular proteins [7]. In the following text, this middle part is called C/EBPβ 3′ UTR element, or simply C/EBPβ 3′ UTR.

Here, we deleted the short sequences, thought to be possible sites of interaction with proteins, in both termini of the C/EBPβ 3′ UTR. Eukaryotic expression plasmids harboring mutated C/EBPβ 3′ UTRs were constructed and stably transfected into SMMC-7721 cells. Then, the malignancy changes in the transfected cell lines were checked by investigating their growth, their ability to form colonies in soft agar, and the tumorigenicity of these transfectants in nude mice. Our results showed for the first time that these deletions in

the C/EBPβ 3′ UTR significantly reduced their tumor suppression activity.

Materials and Methods

Cell lines and animals

SMMC-7721 human hepatoma cell line [8] was from the Cell Bank, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cl1 revertant cells, plasmid pSP64/0.28 [9] and plasmid p14-6 were established, maintained, and stored by our group. Plasmid p14-6, which harbors the middle part of C/EBPβ 3′ UTR cDNA (**Fig. 1**), was from a cDNA library of normal human pcD2 cDNA library by procedures aiming at isolation of clones with anti-oncogenic activity [5]. All cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, USA) supplemented with 10% newborn calf serum (super grade; Si Ji Qing Biotechnological Materials Co., Hangzhou, China) and 100 μg/ml of both ampicillin and streptomycin in cell incubator at 37°C with 5% CO_2. Balb/c *nu/nu* nude mice were purchased from the Shanghai Experimental Animal Core Facility, Chinese Academy of Sciences (Shanghai, China), and were kept under specific pathogen-free (SPF) conditions.

Site-directed mutagenesis and transfection procedures

Site-directed mutagenesis of C/EBPβ 3′ UTR was performed on pSP64/0.28 plasmid using the Gene tailor site-directed mutagenesis kit (Invitrogen) according to the manufacturer's instructions. The plasmids were stably transfected into SMMC-7721 cells using Calcium phosphate transfection kit (Invitrogen) according to manufacturer's instructions, and G418 (Invitrogen) was used as the screening drug.

RT–PCR to confirm existence and expression of plasmids in N0 and N2 transfectants

Two pairs of primers were designed. One pair, P1 and P2, is located on the 0.28 kb cDNA insert and the

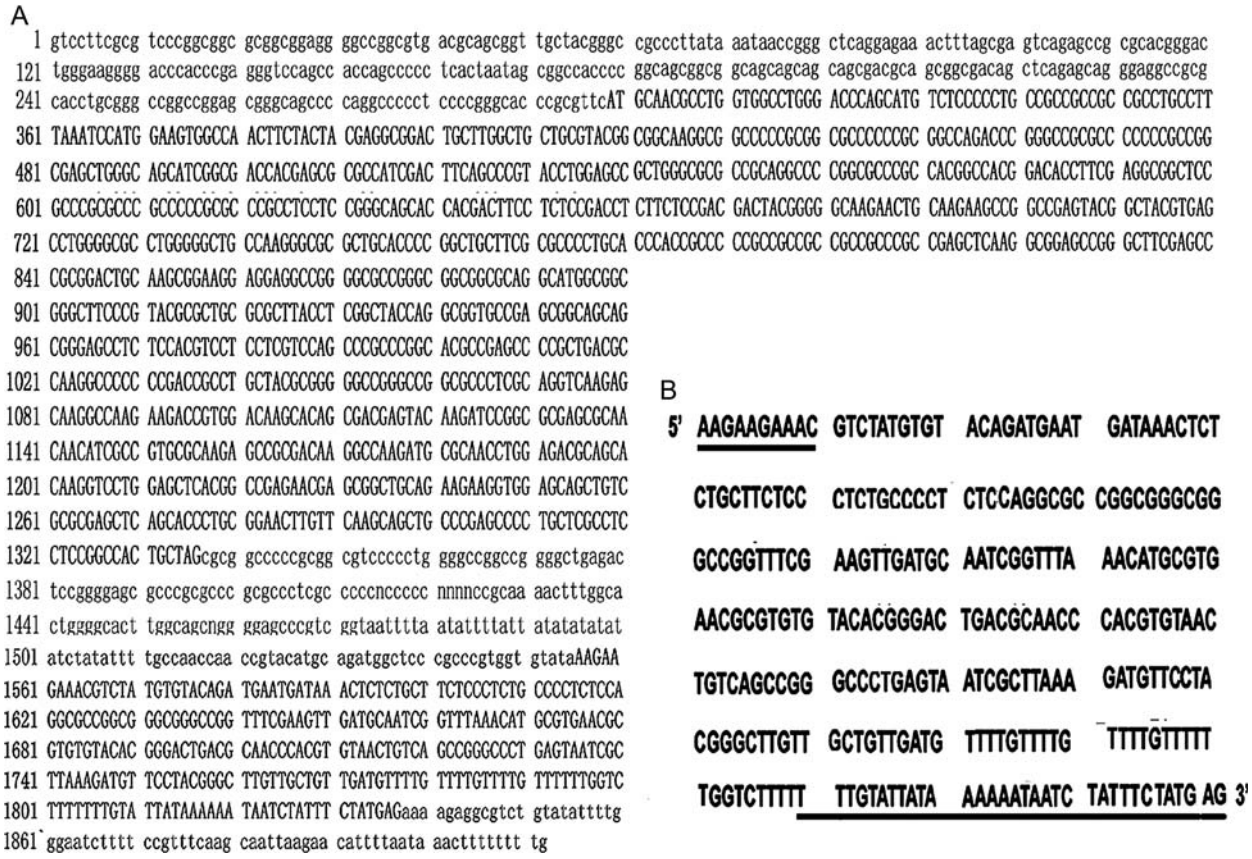

Fig. 1 Sequence of C/EBPβ 3′ UTR cDNA and recombinant plasmid construction (**A**) Sequence of full-length C/EBPβ gene and location of the 3′ UTR fragment used in this work (cloned in p14-6). The coding region is from the base 299 (A) to the base 1336 (G) (totally 1848 bp) and the 3′ UTR fragment is from the base 1556(A) to the base 1837 (G) (totally 282 bp; both regions are in capital letters). (**B**) The 282 base long fragment of the C/EBPβ 3′ UTR cDNA. Underlined regions are to be deleted.

pCMV-script vector, respectively. Another pair, P3 and P4, is located within the neomycin resistance gene of the vector (**Table 1**). The RT was performed with MMLV reverse transcriptase and respective downstream primers.

Cell growth curve and least square curve fitting of curves

The growth curves of cell lines were determined using the method described previously [5]. Briefly, cells were inoculated into 60 mm diameter plates. At defined time, plates were taken out, culture media were completely removed, and cells were suspended in 1.00 ml of 1% Triton X-100. Then, the optical densities at 330 nm were determined and compared with the standard curve plotted by the same method using known amounts of cells.

It is well known that the growth of tumor cell clones and of solid (avascular) tumors complies with the Gompertz rule [10, 11]. It was found that the non-linear least square curve fitting (LSCF) to the polynomial approximation of Taylor series extension of the Gompertz equation gives more accurate curves than the original Gompertz model [12]. Therefore, all curves here were plotted using a program [13] of LSCF to the four-order polynomial, i.e. $y = ax^4 + bx^3 + cx^2 + dx + e$. According to our experience, the four-order polynomial can represent cell growth laws most satisfactorily, and it is very helpful (much better than the common breaking-line graphs) when the growth rules were to be found from a set of dispersed experimental data.

Soft agar colony formation test

The soft agar colony formation test was done according to our previous method [5] with modifications. Briefly, the bottom agar medium was prepared by mixing equal volumes of 1.2% agar at 50°C and 2 × RPMI 1640 medium, and pouring into 3 cm diameter culture plates. The top soft agar was prepared by mixing melted 0.6% agar at 50°C with equal volume of 2× RPMI 1640 and temporarily kept in a 40°C water bath. Cells were harvested by trypsinization, washed twice with 1× RPMI 1640 medium without serum, then suspended in this medium. Cell concentration was determined by cell counting method (count 3–4 times, taking the average value). Then, $1–1.5 \times 10^3$ cells were taken from the cell suspension and quickly mixed with an agar aliquot at 40°C. The mixed agar was poured onto the bottom agar. The plates were moved into the incubator. After 2 weeks, plates were examined under an inverted microscope and colonies larger than 50 cells were counted.

Tumorigenicity in nude mice

All animal experiments were done according to guidelines issued by Shanghai Municipal Administrative Committee for Experimental Animals. The nude mice used were 4–5-week-old males fed and housed under air-conditioned SPF conditions for 1 week before the experiment.

Cells were harvested by trypsinization, washed twice with RPMI 1640 medium without any additives, and resuspended in the same medium. Cell concentration was determined by cell counting method (count 3–4 times, taking the average value). Cells were divided into 150 μl aliquots, each containing 5×10^6 cells, and stored on ice until injection. The cells were injected *sub cutem* into the back of nude mice using a 1 ml syringe, each mouse receiving one injection. All the operations were completed within 2–3 h. Starting from the third day post-injection, the longest diameter (a) and the shortest diameter (b) of the appearing tumors were measured every 3 days with an electronic Vernier Caliper and the tumor volumes were calculated according to the formula: $v = 0.5ab^2$ [14]. At the end of the experiment, the mice were sacrificed and the tumors were dissected, fixed

Table 1 SMMC-7721 and its transfectants used in malignancy testing experiments

Role in experiments	Cell name	Plasmid harbored	Insert of plasmid	Phenotype
Experimental	N2	pCMV-N2	Deleted seq. 1–10 and 249–282	To be tested
Experimental	M3	pCMV-M3	Deleted seq. 249–282	To be tested
Experimental	M5	pCMV-M5	Deleted seq. 1–10	To be tested
Revertant control	Cl1	p14-6	C/EBPβ 3'UTR	Revertant
Empty plasmid transfectant control (to check effect of plasmid itself)	N0	pCMV-script	None	Malignant
Original malignant control	SMMC-7721	None	None	Malignant

with neutral formaldehyde, and subjected to histopathologic analysis.

Statistical analysis

The data were analyzed using two-tailed Student's *t*-test and the *P*-value of <0.05 was considered significant.

Results

Deletion mutagenesis of C/EBPβ 3′ UTR and recombinant plasmids construction

As the tumor suppression activity of C/EBPβ 3′ UTR might be due to its interactions with *trans*-acting factors in the malignant cells, we determined to specifically delete those sequences from C/EBPβ 3′ UTR that might be sites of interaction, rather than deleting its sequences gradually. In our preliminary RNA-protein-binding experiments (data not shown), we found that the nucleotides 1–10, and 249–282 of C/EBPβ 3′ UTR were possible protein interaction sites. Therefore, we performed site-directed mutagenesis to remove nucleotides 1–10, and 249–282, which were at both termini, from the 0.28 kb C/EBPβ 3′ UTR cDNA (**Fig. 1**). To investigate if a single short sequence was enough to lead to the change of tumor suppression activity of C/EBPβ 3′ UTR RNA, we have constructed three deletion mutants of 0.28 kb cDNA: (i) 0.28 kb cDNA with nucleotides 1–10 (near 5′ terminus) deleted; (ii) that with nucleotides 249–282 (near 3′ terminus) deleted; and (iii) that with both deleted. For transfection, the mutated C/EBPβ 3′ UTR cDNA fragments were subcloned into pCMV-script vector (Stratagene, Cedar Creek, USA), forming pCMV-M3 (only sequences 249–282 deleted); pCMV-M5 (only sequences 1–10 deleted); and pCMV-N2 (both deleted).

Successful stable transfection of recombinant plasmids into SMMC-7721 hepatoma cells

In stable transfection of SMMC-7721 cells, we found that this cell line itself possessed a certain resistance ($G418^R$) to the screening antibiotic, G418. Therefore, to avoid getting false transfectants that did not contain plasmids, we had to increase the concentration of G418 to as high as 2 mg/ml after transfection. Under this drug concentration, all the $G418^R$ clones did contain plasmids integrated into cellular genome. However, these clones were obtained at the expense of significant abatement of the number of obtained cell clones. We did not pick single clones but used pooled transfectants in the following experiments, because, according to our experience, individual single cell clones from transfectants by one plasmid are similar in their characteristics. The morphology of these cell lines are all similar [**Fig. 2(A)**].

The existence of recombinant plasmids in the M3, M5, N2, and N0 transfectants and their expression were confirmed by RT-PCR [**Fig. 2(C,D)**].

Experimental system: experimental cells for checking malignant phenotype change and controls

Our experimental system consists of six cell lines. The experimental cell lines, M3, M5, and N2, were derived by transfection of SMMC-7721 cells with pCMV-M3, pCMV-M5, and pCMV-N2, respectively. The following three cell lines are phenotype controls: Cl1, the revertant control; N0, a transfectant with empty pCMV-script; and the original SMMC-7721 cells. The phenotypes of latter two cell lines are malignant and they were used as malignant phenotype controls. Both the vector for p14-6 (namely, pcD2) and the pCMV-script have no potential of malignant transformation [5]. Therefore, the use of the two different vectors did not obscure the experimental results.

Growth rates and colony formation rates: slight differences

Growth rate of many malignant cells are increased compared with their normal counterparts, although exceptions also exist. We measured the growth rates of experimental and control cell lines using a spectrophotometric method [5], and growth curves were shown in **Fig. 2(B)**. The growth curves were drawn with data from one experiment, where at least three dishes were counted for each cell line. Cell growth rates were very similar and the differences between them had no significance statistically, but they did have discernible, although minor, differences, as revealed by curves plotted using LSCF. Cl1 grows most slowly among all four cell lines. In contrast, N0 and SMMC-7721 cells grow most quickly, and their growth curves have two peaks, probably because some cells were overgrown and died even at earlier stages of growth. Notably, N2 has somewhat increased growth rates compared with Cl1. And M3 and M5 both grew more quickly than Cl1, similar with N2.

N2, M3, and M5 have increased anchorage independence in soft agar test

The ability to grow and proliferate in 0.3% agar suspension, without contacting the wall of culture plates, namely anchorage-independent cell growth, is a property of the overwhelming majority of neoplastic cells.

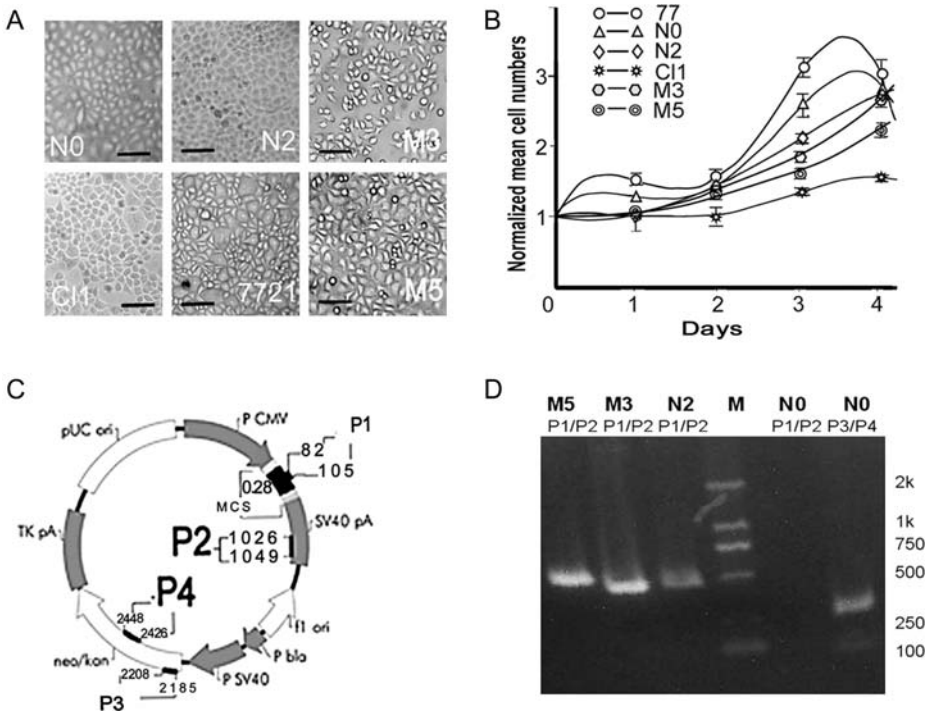

Fig. 2 Characteristics of stable transfectants (**A**) Morphology of the six cell lines. 7721 or 77, SMMC-7721 malignant liver cancer cell line. Cl1, a revertant derived by transfection of p14-6 plasmid into SMMC-7721. N0, a malignant cell line derived by transfection of empty pCMV-script plasmid into SMMC-7721. N2, M3 and M5, the experimental cell lines, derived by transfection of pCMV-N2, pCMV-M3 and pCMV-M5 into SMMC-7721, respectively. (For details of plasmids, see the text. The same abbreviations are used in **Fig. 3** as well.) Bar = 100 μm. (**B**) Growth curves of the six cell lines, normalized to the cell number plated, plotted using the least square curve fitting. These cells were cultured without changing the medium. After 4 days, some cells started to detach from the plate. (**C**) The primers designed to confirm the existence and expression of plasmids in N0 and N2 transfectants. (**D**) The RT–PCR results, showing the expression of the transfected plasmids in the cells.

We performed this soft agar tests to measure the ability of anchorage-independent growth of transfectants and compared it with the control cell lines. Single cells (1000 cells) of each cell lines were allowed to grow in 1 ml of 0.3% soft agar. After 2 weeks, cell colonies of different size and shape developed. Notably, the colony formation rate of N2 is much higher than Cl1, indicating that N2 has stronger malignancy than Cl1 (**Fig. 3**); and M3 and M5 also gave colony formation rates higher than Cl1, and M3 was again higher than M5. Therefore, the mutated C/EBPβ 3′ UTR has reduced tumor suppression activity, and one single short sequence deletion was able to reduce this activity.

Tumorigenicity of N2 is similar with malignant controls

The ability to grow in immunodeficient nude mice is an intrinsic characteristic of cancer cells that directly reflects their malignancy [15]. We investigated this characteristic of the transfectants and compared it with revertant and malignant control cells. The cells (5×10^6 cells) in one injection were injected subcutaneously into the back of one nude mouse; at least six mice were used for each cell line. Daily observation and tumor size measurement on every third day showed that the Cl1 tumors stopped growing shortly after inoculation, and tended to disappear [**Fig. 3(A,B)**]. In contrast, the original malignant control SMMC-7721 cells formed tumors with very diverse sizes; the larger tumors grew continuously and more rapidly [**Fig. 3(A,B)**]. Another malignant control, N0, behaved similarly to the SMMC-7721 cells [**Fig. 3(A,B)**]. The N2 cells behaved differently from Cl1, but very similarly to the malignant controls. The growth rates among tumors of the same line were diverse, and some grew very rapidly. The growth curves also showed that the growth patterns of N2, N0, and SMMC-7721 were very similar with each other, and was very different with Cl1 that was going down. The M3 and M5 cells again gave rise to growing tumors, the growth rates of them were similar with N0, and M3 tumors grew more quickly than M5.

The weights of tumors formed of the N2 cells were compared with those of controls Cl1, N0, and SMMC-7721 cells using the two-tailed Student's *t*-test.

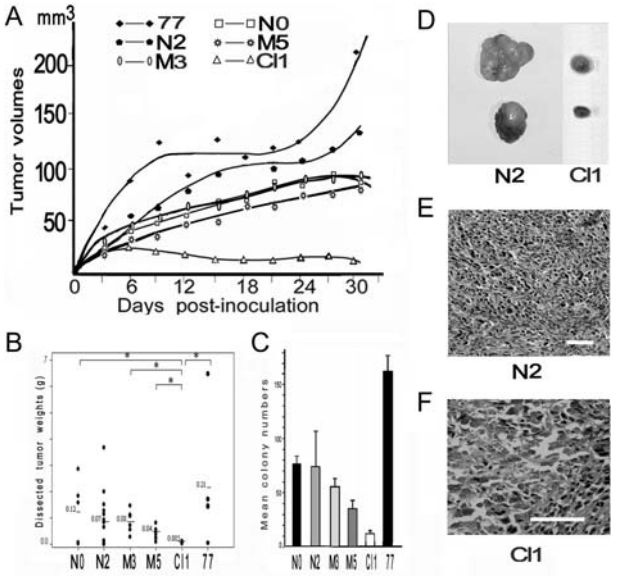

Fig. 3 Malignancy tests of all 6 cell lines (**A**) General growth rules of tumors in nude mice. The tumors were measured with electronic Vernier caliper; the curves were plotted using least square curve fitting approximation of Gompertz model. Therefore, the curves here reflect the specific growth rules of respective tumor types. (**B**) Weights of dissected tumors. Bars indicate average weights. Some tumors' weights are very similar, especially small tumors, therefore their dots overlap. $*P < 0.05$. (**C**) Mean colony numbers per soft agar plate of each cell line. Only colonies containing more than 50 cells were counted. (**D**) Examples of dissected tumors, both large (N2 tumor was taken) and small (Cl1). (**E**) A section of a tumor from N2 cells, showing actively proliferating cells. (**F**) A small tumor section, showing a large number of apoptotic cancer cells (note the fragmented or deformed nuclei). HE staining, Bar = 100 μm.

Similar with the above, the differences of tumor weights between Cl1 and SMMC-7721, N0, M3, and M5 were statistically significant. But the differences between N2 and SMMC-7721, NO, M3, and M5 were not significant.

N2 has weaker apoptosis-inducing effect than Cl1, as shown by histochemistry

The dissected tumors from nude mice were examined by histochemistry. Under the microscope, it was found that all the rapidly growing tumors had actively proliferating cancer cells, while all the tumors that tend to disappear had large numbers of apoptotic cells [**Fig. 3(D–F)**]. Therefore, transfection by unmutated C/EBPβ 3′ UTR (Cl1) induced apoptosis in tumor cells, thereby inhibiting the growth of the tumor. However, the apoptosis-inducing effect of mutated C/EBPβ 3′ UTR (N2) is much weaker. Once again, this indicated that the deletion mutations within the C/EBPβ 3′ UTR reduced the tumor suppression activity of C/EBPβ 3′ UTR.

Discussion

Herein, we present, for the first time, data demonstrating that the tumor suppression activity of the C/EBPβ 3′ UTR is reduced by deletion mutations, suggesting that the deleted sequences were necessary for the tumor suppression activity. Deletion of 10 nucleotides at the 5′ terminus and 33 nucleotides at the 3′ terminus of the C/EBPβ 3′ UTR led to increased tumorigenicity of the transfectants in nude mice, as well as enhanced anchorage-independent growth and colony formation ability; however, these deletions affected cell growth rates only slightly. And, as seen in **Fig. 3**, the tumor suppression activity of the deleted C/EBPβ 3′ UTR is very similar with that of the empty plasmid, indicating that the elements responsible for tumor suppression activity of C/EBP 3′ UTR may just be these two short terminal sequences.

In 1993, Blau's group [1] found that a short RNA from the 3′ UTR of α-tropomyosin has tumor suppression effects, and proposed the concept of the riboregulator to describe a regulator of the normal cellular phenotype and tumor suppressor, and suggested that the continued expression of the 3′ UTR is necessary for tumor suppression. This is consistent with our results, as the C/EBPβ 3′ UTR is highly expressed in its transfectant [**Fig. 2(D)**]. Our results are also consistent with the report that mutations in the 3′ UTR of prohibitin which alone has tumor suppression activity [16], leading to the loss of its antiproliferative activity [17].

So far, besides C/EBPβ 3′ UTR RNA, five other eukaryotic mRNA 3′ UTRs were found to have tumor suppression activity [1–4]. To compare their structures is very interesting. Therefore, we compared the primary structures of all the six 3′ UTRs by using ALIGN software in the NCBI website. We did not find any significant similarity between C/EBPβ 3′ UTR and the five 3′ UTRs. However, when we drew their secondary structures using RNA structure 4.4 software, all of the 3′ UTRs appeared as loops of different lengths ligated by double-stranded stems of 2–12 bp long; although no stem or loop between these six 3′ UTRs is completely identical (data not shown). Therefore, we think that their tumor suppression activity may be related to these secondary structures, which are possible interaction sites with cellular protein factors. It is very interesting that the 30–50 nucleotides at the 3′ terminus of the C/EBPβ 3′

UTR, including those deleted in site-directed mutagenesis in this work, forms a stem-loop structure that exists in all energy conditions possible in the RNA structure software; i.e. it is probably an actually existing stable secondary structure (**Fig. 4**). The deletions just destroyed this stem-loop structure. The sequences that were deleted from C/EBPβ 3′ UTR are possible sites of interaction with cellular protein factors, as suggested by our preliminary study of RNA-protein binding and ultraviolet cross-linking as well. Therefore, we think that the interaction between this stem-loop structure with cellular protein factors may underlie the molecular mechanism of the tumor suppression effect of C/EBPβ 3′ UTR. However, right now we are unable to explain exactly why the deletion of 5′ short sequence also decreased the tumor suppression activity. Other secondary structures that contain this sequence may also play a role.

It is noteworthy that the control plasmid without insert exerted somewhat tumor suppression effect: the N0 cells

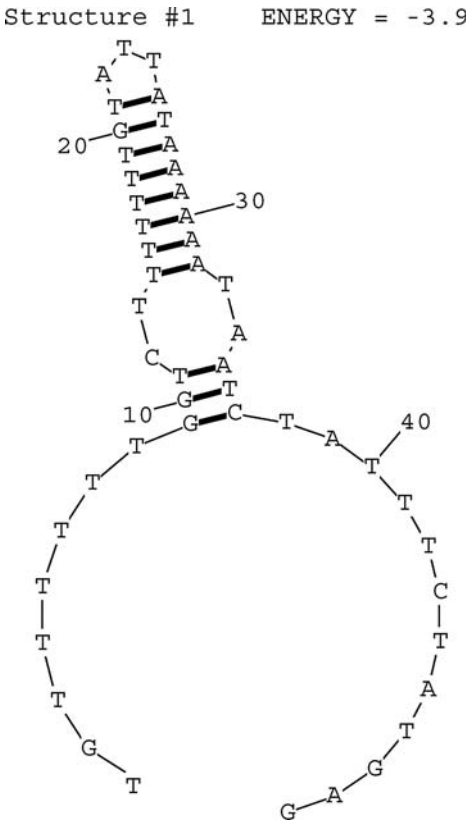

Fig. 4 The representative stem-loop structure of the sequence at 3′-terminus of the C/EBPβ 3′ UTR element, including that deleted in this work It was drawn using RNA structure 4.4 software. The sequence shown is from 233rd through 282nd nucleotides of the element (233rd nucleotide is named 1st in the figure, and so on). The deletion destroys this step-loop structure.

with this empty plasmid appeared less tumorigenic, compared with the 7721 cancer cells [**Fig. 3(A)**]. This may mean that the transfection process or the empty plasmid can somewhat affect the cell growth negatively. To our knowledge, there has been no report on this. This phenomenon emphasizes the importance of a control of non-transfected malignant cell line in the tumorigenicity test, although the suppression effect of the empty plasmid is much weaker than that caused by C/EBPβ 3′ UTR.

In conclusion, this report revealed the importance of the sequences at both termini of C/EBPβ 3′ UTR for the tumor suppression activity. Our findings will assist in the elucidation of the molecular mechanism of tumor suppression and tumorigenesis, which could provide a basis for a novel gene therapy strategy against cancer.

Acknowledgements

We thank Feng Liu and Jinmei Zhang for taking care of nude mice in this article.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (No. 39970172, No. 30540079, and No. 30670449), and the Creation Foundation of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

References

1 Rastinejad F, Conboy MJ, Rando TA and Blau HM. Tumor suppression by RNA from the 3′ untranslated region of alpha-tropomyosin. Cell 1993, 75: 1107–1117.
2 Manjeshwar S, Branam DE, Lerner MR, Bracket DJ and Jupe ER. Tumor suppression by the prohibitin gene 3′ untranslated region RNA in human breast cancer. Cancer Res 2003, 63: 5251–5256.
3 Fan H, Villegas C, Huang A and Wright JA. Suppression of malignancy by the 3′ untranslated regions of ribonucleotide reductase R1 and R2 messenger RNAs. Cancer Res 1996, 56: 4366–4369.
4 Ishiwatari H, Nakanishi K and Kondoh G, *et al*. Suppression of tumor growth by the 3′ untranslated region of mel-18 in 3Y1 cells transformed by the E6 and E7 genes of human papillomavirus type 18. Cancer Lett 1997, 117: 57–65.
5 Liu DG, Noda M, Wang D, Chen ZZ, Ikawa Y and Li ZP. A cDNA clone with antioncogene activity. Sci China 1992, 35: 822–833.
6 Liu DG, Jiang QH, Wei YY, Sun L, Fu BB, Zhao FK and Zhou Q. Gene expression profile favoring phenotypic reversion: a clue for mechanism of tumor suppression by NF-IL6 3′ UTR. Cell Res 2003, 13: 509–514.
7 Liu DG and Sun L. Direct isolation of specific RNA-interacting proteins using a novel affinity medium. Nucleic Acids Res 2005, 33: e132.

8 Dong RC, Zhou RH, Lu FD and Ta WZ. Establishment of SMMC-7721, a human hepatocarcinoma cell line, and observations on its biological characteristics. J 2nd Military Medical University (Shanghai) 1980, 1: 5–9.
9 Liu DG, Li Z, Akira S and Kishimoto T. Overexpression of a reversion-related protein in the revertant RR Cl1s. Sci China C Life Sci 1996, 39: 300–309.
10 Bi YL, Gao JC and Yao M. Nude mice Wilms' tumor model. Chin J Pediatr Surg 2000, 21: 115–118.
11 Winsor CP. The Gompertz curve as a growth curve. Proc Natl Acad Sci USA 1932, 18: 1–8.
12 Yin Y, Xu JH and Duanmu JS. Parameter estimation for modified Gompertz model based on the least-square-method of nonlinear regression. J Air Force Engineering University, PLA, China (Natural Science Edition) 2005, 6: 77–79.
13 Manczur A. Komputerowy program metoda najmniejszych kwadratów, http://www.prz.rzeszow.pl/~janand/mnk.htm 2007.
14 Sun L, Fu BB and Liu DG. Systemic delivery of full-length C/EBPβ/liposome complex suppresses growth of human colon cancer in nude mice. Cell Res 2005, 15: 770–776.
15 Fogh JM, Fogh T and Orfeo J. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J Natl Cancer Inst 1977, 59: 221–226.
16 Manjeshwar S, Branam DE, Lerner MR, Brackett DJ and Jupe ER. Tumor suppression by the prohibitin gene 3′ untranslated region RNA in human breast cancer. Cancer Res 1996, 639: 5251–5256.
17 Jupe ER, Liu XT, Kiehlbauch JL, McClung K and Dell'Orco RT. Prohibitin in breast cancer cell lines: loss of antiproliferative activity is linked to 3′ untranslated region mutations. Cell Growth Differ 1996, 7: 871–878.