Upstream binding factor up-regulated in hepatocellular carcinoma is related to the survival and cisplatin-sensitivity of cancer cells

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ABSTRACT

Upstream binding factor (UBF) is an RNA polymerase I-specific transcription factor. By representational difference analysis, Northern blot, and cDNA array analysis, up-regulation of UBF was detected in 12 of 17 clinical hepatocellular carcinoma samples comparing to the paired normal liver tissues. Introduction of UBF in human lung fibroblast cells that do not express UBF resulted in an accelerated rate of cell growth; on the other hand, antisense oligodeoxynucleotides (ODNs) treatment of UBF-expressing hepatoma cell lines reduced the level of UBF protein, suppressed the colony formation capacity of these cells on soft agarose, and finally caused cell death. Annexin V binding analysis suggested that anti-UBF ODN-caused cell death might involve weak apoptosis, however, DNA laddering and cleavage of poly (ADP-ribose) polymerase were not observed in these ODN-treated cells. Expression profiling of the anti-UBF ODN-treated cells using a human cDNA array revealed that the expression of 30 genes was altered in response to the inhibition of UBF expression. Notably, UBF expression could increase the cell sensitivity to the chemotherapeutic reagent cis-diaminedichloroplatinum (II). We proposed that UBF is fundamental to the survival of cells expressing the gene, and is potential as a target for screening anti-cancer drugs and an indicator in selecting chemotherapeutic reagents.—Huang, R., Wu, T., Xu, L., Liu, A., Ji, Y., Hu, G. Upstream binding factor up-regulated in hepatocellular carcinoma is related to the survival and cisplatin-sensitivity of cancer cells. FASEB J. 16, 293–301 (2002)

Key Words: representational difference analysis · UBF · HCC · antisense oligodeoxynucleotide · cisplatin

Upstream binding factor (UBF) is an RNA polymerase I-specific transcription factor (1) recognizing the ribosomal RNA gene promoter together with the transcription factor SL1 and facilitating the transcription of 5.8S, 18S, and 28S rRNAs (2). It is also known as the nucleolus organizer region (NOR) autoantigen N90-90 detected by antisera from patients with autoimmune diseases, such as systemic sclerosis (4).

Evidence suggests a correlation between UBF and cell proliferation. The accumulation of UBF is directly related to RNA polymerase I activity, inversely related to cell doubling time, and might represent a cytopathologic parameter of proliferating rate of cancer cells (5). Argyrophilic nucleolar proteins, including UBF, accumulate in highly proliferating cells and are indicated to be a cell proliferation marker with possible prognostic value for several types of human cancer (6). Measurement of active NORs during mitosis based on staining for UBF (7) is a good alternative for detecting the ribosomal activity in malignant tissues (6). Transcription of UBF gene could be inhibited by the retinoblastoma susceptibility gene product Rb (8) and DNA binding activity of the UBF protein could be affected by the binding of Rb (9). Phosphorylation is a primary mechanism by which the activity of UBF is modified (10). It is catalyzed by the casein kinase II (CKII) at serine residues in the carboxy-terminal acidic domain (11) and by G1-specific cdks (cdk4-cyclinD1/cdk2-cyclinE) at Ser484 (12), required for the growth-depenent control of rRNA synthesis. However, the detailed molecular mechanism through which UBF affects cell proliferation is largely unknown.

UBF exhibits an extraordinarily high binding activity to cisplatin [cis-diaminedichloroplatinum (II)] and its DNA lesions (13). Cisplatin has been widely used as a chemotherapeutic agent for testicular, ovarian, head and neck, and small cell lung cancers (13, 14). One mechanism of cisplatin therapy is through the formation of cisplatin-DNA adducts, which may hijack UBF and cause the functional inhibition of UBF (15). HCC is a common and highly malignant tumor and ranks fifth in frequency in the world, with an estimated 427,000 new cases in 1990. Worldwide HCC affects ~250,000 to 1,000,000 individuals annually and causes at least 200,000 deaths per year. The geographic areas at highest risk are eastern Asia and middle and western
Hepatitis B virus infection and aflatoxin B1 exposure are two major etiological factors associated with HCC, followed by hepatitis C virus infection, chronic alcohol exposure-induced liver disease, and primary biliary cirrhosis (17).

Here we report that UBF was up-regulated in ~70% of clinical HCC samples compared to paired nontumorous liver samples and that inhibition of UBF expression in human hepatoma cell lines resulted in cell death. Evidence is also presented that UBF might be related to the sensitivity of cancer cells to cisplatin. A role for UBF in regulation of cell survival and hepatocarcinogenesis is suggested.

MATERIALS AND METHODS

Clinical samples

Clinical tumor samples and paired nontumorous tissue samples from the same patients, including liver, lung, esophagus, breast and ovary tissues, were collected immediately after surgery from Zhongshan Hospital. All tissues were verified by pathological examination.

cDNA representational difference analysis (RDA)

RDA was carried out as reported previously (18, 19) with modifications. Poly (A)+ mRNA was isolated from paired HCC tissues using an oligotex mRNA isolation kit (Qiagen, Hilden, Germany) and converted to cDNA by reverse-transcription using biotinylated primer PNotIT16 (Ransom Hill, Ramona, CA) and AMV reverse transcriptase (Promega, Madison, WI). cDNA was digested with Hsp92II restriction enzyme, ligated to double-stranded adapters generated by annealing of biotinylated primers PA and PB (for tester) or PC and PD (for the driver), and amplified using primers PNotI and PB for the tester or PSP6 and PD for the driver. The first round of subtraction was performed by denaturing and reannealing the driver and tester amplicons in a ratio of 100:1, and removing the tester using streptavidin-coated magnetic beads (Promega). The tester was then eluted and amplified using primers PNotI and PB. The PCR product was further subtracted by the driver amplicons twice in a ratio of driver: tester = 104:1 and 106:1, respectively. The final PCR product was digested with NotI and Hsp92II and cloned into the pGEM-5Z (+) (Promega). Primers used in this procedure are listed in Table 1.

Northern blot analysis

30 µg of total RNA prepared by Trizol (GIBCO BRL, Grand Island, NY) was separated using 1% agarose gel, transferred onto Hybond-XL nylon membrane (Amersham Pharmacia, Buckinghamshire, England), and fixed to the membrane by baking at 80°C for 2 h. Nylon membranes were prehybridized with 5 ml prehybridization solution (6×SSC, 0.5% SDS, 5×Denhardt, 100 µg/ml denatured salmon sperm DNA) at 65°C for 3 h. Overnight hybridization with the 32P-labeled UBF cDNA in 3 ml hybridization solution (6×SSC, 0.5% SDS, 100 µg/ml salmon sperm DNA) was followed by stringent washing (0.1×SSC, 0.5% SDS, 65°C, 1 h). Results of hybridization were visualized by a phosphor screen scanned with an FLA-3000A Plate/Fluorescent Image Analyzer (Fuji Photo Film, Tokyo, Japan).

Gene expression profiling

cDNA array was assembled from 14,000 cDNA clones spotted on 16 × 12 cm Hybond-N nylon membranes (Amersham Pharmacia). Isolation of poly (A)+ mRNA, labeling probes, hybridization, and data analysis were described previously (20).

Cell culture

Cultured cell lines, including hepatoma cell SMMC-7721, BEL-7402 and BEL-7404, lung fibroblast HLF, gastric cancer cell MKN45, neuroblastoma cell SK-N-SH, liver cell L02, lung carcinoma cell A549, and melanoma cell A375, were all of human origin and purchased from Type Culture Collection of Chinese Academy of Sciences, Shanghai. These cells were cultured in Dulbecco’s modified Eagle’s medium (GIBCO BRL) containing 10% fetal bovine serum, 100 µg/ml strep-

<table>
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<tr>
<th>Primer set</th>
<th>Name</th>
<th>Sequence</th>
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<tr>
<td>Tester</td>
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<td></td>
<td>PA</td>
<td>5’-TAA TAC GAG TCA CTA TAG GGC GAC ATG-3’</td>
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<tr>
<td></td>
<td>PB</td>
<td>5’-TCG CCC TAT AGT G-3’</td>
</tr>
<tr>
<td></td>
<td>PNotI</td>
<td>5’-Biotin-AAT AGT TAG CGG GCG CCT GA-3’</td>
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<tr>
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<td>5’-GAA GAC GCC AAA-3’</td>
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tomycin sulfate, and 100 units/ml penicillin at 37°C in 5% CO₂.

Plasmid construction and transfection

The coding region of the UBF gene was amplified from BEL-7402 cell cDNA using primers PDS and PDA (Table 1) and cloned into pcDNA3 vector (Invitrogen, Carlsbad, CA). Transfection of pcDNA3-UBF into HLF cells was carried out using the calcium phosphate method (21); stable UBF-transfected cells were selected in the presence of 800 μg/ml G418 for 6 wk.

Growth rate measurement

Growth rate of cultured cells was measured by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] method. Briefly, 5 × 10⁵ cells were seeded per well in 96-well plates, incubated until 20 μl MTT stock solution (5 mg/ml in 0.1 M PBS) was added to each well, followed by incubation at 37°C for 4 h. The reaction was terminated by aspirating the medium. In each well 150 μl DMSO was added to dissolve the formazan crystals; plates were read at 540 nm on a microplate reader (Bio-Tek, Winooski, VT).

Colony formation assay

Anchorage-independent growth capacity of cultured cells was determined by measuring the colony-forming efficiency in soft agarose. Experiments were conducted using 6-well plates containing a 2 ml feeder layer [0.6% low gelling temperature agarose (Sigma, St. Louis, MO) in medium] and a 1 ml top layer with 1 × 10⁵ cells in 0.35% low gelling temperature agarose in medium. Fresh medium was supplied weekly. After 14 days, cells were stained by MTT overnight and colonies were counted.

Antisense phosphorothioate oligodeoxynucleotides (ODNs)

Two UBF-specific antisense (AS) ODNs and other control ODNs were designed as shown in Table 1 and phosphorothioate ODNs were synthesized by Sangon (Shanghai, China). ODNs were freshly dissolved in 1M PBS before use and transfected with 20 μg/ml LipofectAMINE Reagent (GIBCO BRL).

Western blot analysis

Cellular proteins (100 μg) were run on 10% SDS-PAGE, electrophoretically transferred to a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany), and blotted using mouse anti-UBF monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or mouse anti-PARP monoclonal antibody (PharMingen, San Diego, CA) (1:1000). After binding an HRP-anti-mouse-IgG antibody (1:1000) (Amersham Pharmacia), Western blot was visualized using the ECL luminescent system (Amersham Pharmacia). The blot was reprobed by goat anti-actin polyclonal antibody (Santa Cruz) (1:5000) according to instructions by Amersham.

Apoptosis and cell cycle assay

Annexin V-FITC binding and propidium iodide (PI) staining were performed according to the manufacturer’s protocol (Roche, Indianapolis, IN). Briefly, 1 × 10⁶ cells were washed with PBS and resuspended in binding buffer. The cells were incubated with 0.25 μg/ml FITC-conjugated annexin V and 10 μg/ml PI, then analyzed by flow cytometry (Becton Dickinson, San Jose, CA). For assay DNA content of cultured cells, both adherent and the floating cells were collected and fixed in ice-cold 70% ethanol, then digested with 50 μg/ml RNase A. After being stained with 1% PI at 37°C for 30 min, cells were analyzed using flow cytometer.

Cell viability assay

Stock solutions of 10 mg/ml cis-/trans-diaminedichloroplatinum (II) (CDDP/TDDP) (Sigma) were prepared in N,N-dimethylformamide and stored at −20°C. Alternatively, CDDP and TDDP were dissolved in sterile PBS to the concentration of 1 mM. Exponentially growing cells were treated with CDDP or TDDP for 24 h, washed, and cultured in drug-free medium for an additional 24 h. Cell viability was evaluated by MTT assay.

RESULTS

Up-regulation of UBF expression in HCC

With a modified RDA method, we were able to enrich the cDNA fragments differentially expressed in a clinical HCC sample from its paired distal liver tissue. The HCC sample was from a 59-year-old Chinese male patient positive for AFP, cytokeratin 8, HBsAg, CD34, proliferating cell nuclear antigen, and proliferation-associated antigen Ki-67, but negative for cytokeratin 7 and HBCAg. Cloning and sequencing of the enriched cDNA fragments revealed that UBF was up-regulated in the HCC tissue.

Northern blot and cDNA array assay were used to determine the expression of UBF in independent HCC samples and their paired normal liver tissues. In 11 of 16 other HCC samples, UBF was found to be up-regulated; in the remaining five HCC samples UBF was either not detected or expressed at levels similar to paired liver tissues (Fig. 1A and Table 2). In contrast, consistent up-regulation of UBF was not detected in 16 paired lung, esophagus, breast, and ovary cancers (Fig. 1B) by Northern blotting, indicating that UBF up-regulation is HCC specific.

Expression levels of UBF were further examined in nine cell lines by Western blotting. UBF was highly expressed in most cell lines, including all three hepatoma lines tested (SMMC-7721, BEL-7402, and BEL-7404), and at a modest level in the liver cell line L-02. UBF expression was not detected in the lung fibroblast line HLF or gastric cancer cell line MKN45 (Fig. 1C).

Introduction of UBF results in accelerated cell growth

To determine the effect of UBF expression on cell proliferation and tumorigenesis, the UBF expression construct was introduced into the lung fibroblast line HLF, which does not express UBF. As shown in Fig. 2A, UBF expression in transfected HLF cell line was confirmed by Western blotting using monoclonal mouse anti-UBF antibody. Although the morphological ap-
pearance of the UBF-transfected cells was indistinguishable from that of parental HLF cells (data not shown), three randomly chosen clones of transfected cells proliferated much faster than the mock (vector-transfected) and parental cells detected by MTT assay (Fig. 2B), with no significant change in cell cycle distribution patterns (Fig. 2C). The ability of colony formation of the UBF-transfected cells was slightly increased compared with the mock and parental cells (Fig. 2D). Thus, UBF appears to confer accelerated growth and elevated colony formation capacities on soft agarose of cells that normally do not express UBF.

Inhibition of UBF expression leads to cell death

To examine the effect of down-regulation of UBF expression on cell lines, an AS approach (22–29) was used. Antisense ODNs complementary to two different regions of the UBF mRNA were designed: AS-1 and AS-2. Control ODNs were also used: missense (MI) ODNs, which are similar to the AS ODNs except for a few mismatch nucleotides; sense (SE) ODNs, which are complementary to AS sequences, and shuffled (SH) ODNs, composed of the same nucleotides as the AS ODNs but in a shuffled order. Since AS-1 and AS-2 had the same effects on the treated cells, only the data of AS-1 ODN are shown.

The cellular uptake of ODNs was demonstrated by the SE ODNs labeled with fluorescein. Using confocal microscope, we observed that the fluorescein-labeled ODNs were taken up in 70%–80% cells at 48 h (data not shown). Anti-UBF ODNs treatment inhibited the proliferation of SMMC-7721 cells that expressed UBF in a dose-dependent manner, whereas the same ODNs had no detectable effect on MKN45 cells, which did not express UBF (Fig. 3A). The AS and control ODNs were applied to hepatoma cell lines SMMC-7721 at a concentration of 5 μM; expression levels of UBF mRNA were measured by cDNA array. The expression intensities of UBF in SMMC-7721 cells treated by AS, SE, SH, MI ODNs, and PBS were 5.49, 16.33, 12.01, 11.62, and 18.96, respectively. (In our cDNA array system, the gene with expression intensity below 10 is defined not to be expressed.) Western blot assay confirmed that UBF protein was depleted in AS ODN-treated cells but not in control ODN-treated cells (Fig. 3B). Thus, AS ODNs specifically inhibit the expression of UBF at both the mRNA and protein level.

Examination of hepatoma cells SMMC-7721 and BEL-7404 at different times after AS ODNs treatment at a concentration of 5 μM revealed that AS ODNs caused morphological changes initially, including increased nucleocytoplasmic ratios (Fig. 3C), then decreased cell numbers (Fig. 3D), finally causing cell death. Similarly, colony formation capacities on soft agarose of SMMC-7721 cells and BEL-7404 cells, which were tumorigenic in nude mice (30, 31), were completely eliminated by

TABLE 2. Expression intensities of UBF in nine paired HCC samples detected by cDNA arraya

<table>
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<th>Sample</th>
<th>Tumor</th>
<th>Normal</th>
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a The data shown mean the signal intensity mean in the cDNA assay. The gene with expression intensity below 10 is defined not to be expressed. Ratio values above 2 were considered significant.

b Divided by zero.

ODNs, which are similar to the AS ODNs except for a few mismatch nucleotides; sense (SE) ODNs, which are complementary to AS sequences, and shuffled (SH) ODNs, composed of the same nucleotides as the AS ODNs but in a shuffled order. Since AS-1 and AS-2 had the same effects on the treated cells, only the data of AS-1 ODN are shown.

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AS ODNs treatment at a concentration of 10 μM (Fig. 3E). These findings suggest that continuous UBF expression in tumor cells is required for their survival and tumorigenesis.

**Mechanism of the UBF inhibition-induced cell death**

The mechanism of AS ODN-caused cell death was investigated. We analyzed the anti-UBF ODN-treated SMMC-7721 cells for annexin V binding, PARP activity, and DNA laddering to determine whether cell death was occurring through apoptosis. Early in apoptosis, annexin V, a membrane-impermeable protein, may be detected on the outer leaflet of the plasma membrane due to the translocation of phosphatidylserine from the inner to the outer leaflet (32). It was observed via flow cytometry that FITC-labeled annexin V binds to 40% of the SMMC-7721 cells after incubation with 5 μM AS ODNs.

**Figure 2.** Effects of UBF expression on HLF cells. A) Western blot assay of total proteins from UBF-transfected, vector-transfected, and parental HLF cells using mouse anti-UBF monoclonal antibody. Protein weight marker is the BENCHMARK prestained protein ladder (GIBCO BRL). B) MTT assay of UBF-transfected, vector-transfected (mock), and parental HLF cells. Bars, sd. C) Cell cycle assay by FACS analysis. D) Colony numbers of UBF-transfected, mock and parental HLF cells in a soft agarose assay. Bars, sd. P1–3, three independent UBF-transfected HLF cell clones; HLF, parental HLF cells; N1–2, two independent vector-transfected HLF cell clones.

**Figure 3.** Effects of anti-UBF ODNs on cultured cells. A) Dose-dependent inhibition of anti-UBF ODNs on the proliferation of SMMC-7721 cells. Bars, sd. B) Western blot assay of total proteins from SMMC-7721 cells treated with ODNs using mouse anti-UBF monoclonal antibody. C) H&E staining of ODN-treated SMMC-7721 cells. Bar size = 15 μm. D) Ratio of cell numbers between ODN-treated and vehicle-treated cells in the first 3 days of ODN treatment. HLF, parental HLF cells; P1, one UBF-transfected HLF cell clone; N1, one vector-transfected HLF cell clone. Bars, sd. E) Colony numbers of SMMC-7721 and BEL-7404 cells treated with ODNs in a soft agarose assay. Bars, sd. AS, antisense ODN-treated cells; SE, sense ODN-treated cells; SH, shuffled ODN-treated cells; MI, missense ODN-treated cells; C, vehicle-treated cells.

UBF IS RELATED TO HCC AND CISPLATIN SENSITIVITY

297
ODNs for 48 h, significantly more than that in control cells; moreover, PI staining indicated that 16% of the AS ODN-treated cells were dead (Fig. 4A). Genomic DNA isolated from the anti-UBF ODN-treated cells was partially smeared, but not the DNA ladders (Fig. 4B). PARP, a 116 kDa nuclear chromatin-associated enzyme usually cleaved into 85 kDa and 25 kDa fragments in apoptotic cells (33), was not cleaved in anti-UBF ODN-treated cells (Fig. 4C). FACS examination showed that DNA in the AS ODN-treated SMMC-7721 cells was weakly degraded (Fig. 4D); however, the influence of AS ODNs on cell cycle progression was not distinct, as no G1, S or G2/M phase accumulation was observed at 24, 48, or 72 h (Fig. 4D, data of 48 h). These analyses suggested that anti-UBF ODN-caused cell death might involve weak apoptosis.

Expression profiling, using a cDNA array representing 14,000 human gene/cDNA clusters (20), was used to further explore the molecular mechanism of anti-UBF ODN-induced cell death. Thirty genes exhibited the altered expression of > twofold between AS ODN-treated cells and control ODNs and untreated cells (Fig. 5). A few genes up-regulated in HCC or other types of tumors such as actin-related protein 2 (Arp2) (Dr. Lijian Hui, personal communication) and muscle-type acylphosphatase 2 (ACYP2) (34) were reduced in anti-UBF-treated hepatoma cells, indicating revised tumor markers resulted from the inhibition of UBF expression. Expression of the genes down-regulated in apoptosis cells, such as ribosomal protein S20 (RPS20) (35) and cytochrome c (HCS) (36), were decreased in AS ODN-treated cells, consistent with cytological observations.

**Cells expressing UBF had higher sensitivity to cisplatin**

Since UBF binds to cisplatin and its DNA adducts, the importance of UBF in carcinogenesis was further explored by testing the sensitivities of cells with different expression levels of UBF to cisplatin measured with the IC$_{50}$ (50% inhibition concentration) values of cell death. As summarized in Fig. 6, the IC$_{50}$ values to CDDP of the cells highly expressing UBF (including

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**Figure 4.** Mechanism of anti-UBF ODN-induced cell death. A) Annexin V binding assay in SMMC-7721 cells treated with ODNs. Viable cells were neither annexin V nor PI positive; apoptotic cells were stained only with annexin V (PI negative); nonviable cells were labeled with both PI and annexin. V, viable cells; N, nonviable cells; A, apoptotic cells. B) Genomic DNA isolated from ODN-treated SMMC-7721 cells using PARP-specific mouse antibody. C) Western blot of total proteins in ODN-treated SMMC-7721 cells using PARP-specific mouse antibody. D) Cell cycle assay by FACS analysis of SMMC-7721 cells treated with ODNs for 48 h. AP, UV-induced apoptotic cells; AS, antisense ODN-treated cells; SE, sense ODN-treated cells; SH, shuffled ODN-treated cells; MI, missense ODN-treated cells; C, vehicle-treated cells; M, 125 bp ladder (Sigma).

**Figure 5.** Illustration of genes responding to anti-UBF ODN treatment in SMMC-7721 cells represented in the cDNA array. Genes are listed in rows. The ratio of gene expression levels in AS ODNs to ones in control ODNs or vehicle is color coded. The differential expression fold is indicated in the color key. AS, antisense ODN-treated cells; SE, sense ODN-treated cells; SH, shuffled ODN-treated cells; MI, missense ODN-treated cells; C, vehicle-treated cells.
SMMC-7721, BEL-7404) were at least threefold lower than those of the cells not expressing UBF, such as MKN45 and HLF cells. The IC_{50} value of anti-UBF ODN-treated SMMC-7721 cells to CDDP was increased ~threefold compared with the parental SMMC-7721 cells; both independent UBF-transfected HLF clones had decreased IC_{50} values vs. the parental HLF cells. However, IC_{50} values of TDDP, an analog of CDDP used as a control, were > 50 μM in all tested cells.

**DISCUSSION**

**UBF is essential to the tumorigenicity and survival of a fraction of HCC**

Using a variety of methods, including modified cDNA RDA, Northern blotting, and expression profiling, we showed that UBF was up-regulated in ~70% of clinical HCC samples compared with paired distal liver tissues from the same patients. Although UBF was not expressed in all HCC samples, anti-UBF ODNs eliminated the tumorigenicity of the cells expressing UBF and caused cell death in a mechanism that may be weak apoptosis. The antisense ODN-mediated inhibition was effective not only in hepatoma cells but also in other cells that express UBF, such as UBF-transfected HLF cells (Fig. 3D). These findings suggest a critical role of UBF in the survival, growth and tumorigenicity of tumor cells that express UBF.

The observation that UBF was expressed in a high percentage of HCC but not in other cancer types suggests that the involvement of UBF in carcinogenesis is cell type dependent. Because overexpression of UBF in cells that normally do not express UBF accelerated the growth rate, the growth control mechanism involv-

ing UBF might include additional factors not present in every cell type.

Accumulation of UBF mRNA and protein might result from the up-regulated transcription in a mechanism similar to that of c-myc (10) or other mechanisms (37–39); the mechanism by which UBF is involved in cell survival is unclear. It was reported that CD95 (Fas/APO-1) -mediated T cell apoptosis was associated with substantial cleavage of a subset of nuclear autoantigens, including UBF (40, 41). The DNA binding activity of UBF can be severely compromised by interacting with Rb (8, 42) either directly or indirectly (9). Furthermore, the X protein of hepatitis B virus may form a specific complex with Rb and thus affect the expression of UBF (44). LOH of Rb was found in 25–48% of HCC cases and Rb gene expression was strongly down-regulated in 30–50% of tumors (44); meanwhile, we found UBF was up-regulated in ~70% of clinical HCC samples. It would be interesting to determine whether there is a correlation between the expression of UBF and Rb gene mutation and abnormal expression.

**UBF and related gene as potential targets for screening of novel anti-cancer drugs**

We observed that inhibition of UBF expression could cause the death of cancer cells and therefore suggest that UBF and the genes cofunctioning with UBF in controlling cell survival could be potential targets for screening anti-cancer drugs. Expression profiling revealed that 30 genes responded to UBF inhibition. Among the up-regulated genes, FBOX3 (F-box only protein 3) was the component of modular E3 ubiquitin protein ligases involved in phosphorylation- and ubiquitin-dependent proteolytic pathways (45); HTGN29, with an accession number of AF226055, was a novel gene expressed in nontumorous human liver cells. Down-regulation of RPS20 and HCS might be related to cell death, as they were involved in apoptosis (35, 36). Reduced expression of Arp2 and ACYP2 genes might indicate an altered cancer phenotype, as it was reported they were involved in HCC and the metastatic phenotype (34), respectively. Hypoxia-inducible gene 1 (HIG1) might be induced by hypoxia or glucose deprivation (46), which occurred often in clinical HCC; hepatitis C-associated microtubular aggregate protein p44 (MTAP44) was associated with the liver microtubular aggregation caused by hepatitis C or hepatitis D virus infection (47). Study of these genes, especially the novel genes responding to UBF inhibition, is warranted to not only further explore the role of UBF in cell death and proliferation control, but also seek novel drug targets.

**Potential of UBF as an indicator for chemotherapy**

The existence of natural or acquired resistance for cisplatin is a major clinical problem (48). It was reported that only 52% of HCC patients were sensitive to
cisplatin as measured by a succinate dehydrogenase inhibition test (49). Possible mechanisms responsible for this resistance included reduced drug accumulation, increased detoxification of cisplatin in the cellular cytoplasm, and increased DNA repair in the cell nucleus (50). Our results suggest an additional explanation. It was indicated that a proportion of clinical HCC did not express UBF, and cisplatin at a clinically achievable plasma level of 10 μM (51) had little effect on cells not expressing UBF. In addition, both UBF-expressing cells and UBF-transfected cells were sensitive to cisplatin; however, when UBF expression was inhibited by AS ODNs, the cells became less sensitive to the drug. Therefore, testing the correlation between the UBF expression and cisplatin sensitivity in clinical cancer samples may help to predict drug sensitivity in chemotherapy. Screening for anti-UBF compounds may lead to the discovery of novel cisplatin-like anti-cancer drugs.

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UBF IS RELATED TO HCC AND CISPLATIN SENSITIVITY