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Cyr61 Is Up-Regulated in Prostate Cancer and Associated With the *p53* Gene Status

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ABSTRACT

Cysteine-rich 61 (Cyr61) is a member of the CCN protein family that has been implicated in diverse biological processes such as cell adhesion, proliferation, angiogenesis, and tumorigenesis. Altered expression of Cyr61 is found to be associated with human cancers. Here we show that Cyr61 was up-regulated in prostate cancer cell lines and tumor tissues. A significant correlation of Cyr61 expression was found between benign prostatic hyperplasia and prostate cancer ($P = 0.002$). However, there was no significant correlation between levels of PSA and Cyr61 expression ($P = 0.2$). Cyr61 may represent an independent prostate cancer biomarker and potentially a useful therapeutic target for prostate cancer treatment. In addition, our analysis based on published data and data present in this report indicted that levels of Cyr61 expression associated with the status of the tumor suppressor gene *p53* in 32 cancer cell lines analyzed, high levels of Cyr61 expression were found in cell lines with mutant or null *p53* gene, whereas lower expression levels of Cyr61 in the cell lines with wild-type *p53*. We further show that over-expression of dominant negative p53 or down-expression of endogenous wild-type p53 resulted in up-regulation of Cyr61 expression, suggesting a functional link between Cyr61 and p53 in cancers. J. Cell. Biochem. 106: 738–744, 2009.

KEY WORDS: CCN FAMILY PROTEIN; Cyr61; BPH AND PROSTATE CANCER; p53

Cysteine-rich 61 (Cyr61) is the first member cloned in the CCN protein family, which includes Cyr61/CCN1, CTGF/CCN2 (connective tissue growth factor), Nov/CCN3 (nephroblastoma-over-expressed), Wnt-1 induced secreted protein 1 (Wisp-1/CCN4), Wisp-2/CCN5, and Wisp-3/CCN6 [Perbal et al., 2003; Leask and Abraham, 2006]. The human Cyr61 gene is located in the short arm of chromosome 1 (1p22-31) encoding a protein with a molecular mass of 42 kDa, it shares a common structural similarity among the CCN family proteins which are conserved across species [Jay et al., 1997; Unoki et al., 2003; Leask and Abraham, 2006]. Cyr61 has been implicated in diverse biological processes such as cell adhesion, proliferation, angiogenesis, and tumorigenesis [Perbal, 2004; Chen and Du, 2007].

Increasing evidence suggests that Cyr61 plays a role in tumorigenesis. Its over-expression has been reported in advanced breast cancer, gliomas, pancreatic and gastric cancers [Tsai et al., 2002; Chang et al., 2004; Xie et al., 2004a,b; Holloway et al., 2005; Lin et al., 2005; Nishigaki et al., 2005; Kwon et al., 2007]. In contrast, down-regulation of Cyr61 has been observed in lung cancer and the levels of Cyr61 expression is correlated with clinical feature of the disease [Chen et al., 2007]. Cyr61 is down-regulated in human hepatocellular carcinoma (HCC) and suppresses cell proliferation and anchorage-dependent growth when it over-expressed in HCC cell lines [Feng et al., 2008].

This paradoxical expression profile in different type of tumors suggests that regulation of Cyr61 expression may be cell type specific and Cyr61 may have multifunctional roles in different cellular context. Consistently, Cyr61 has been suggested to act through direct binding to distinct integrins, which mediate Cyr61 functions in a cell type- and context-dependent manner [Lau and Lam, 1999; Chen and Lau, 2008].

Although Cyr61 was originally reported to be down-regulated in prostate cancer [Pilarsky et al., 1998], more recent studies suggest that its expression is significantly increased in benign prostatic

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hyperplasia (BPH) and it promotes prostatic stromal and epithelial cell proliferation [Sakamoto et al., 2003, 2004a,b]. Expression of Cyr61 in prostate tumors has not been examined and its role in prostate cancer progression remains elusive.

The high incidence of prostate cancer and the lack of effective therapies for late stage diseases make its early detection extremely important. The prostate-specific antigen (PSA) has been used clinically as a diagnosis biomarker. However, imperfect correlation with cancer hinders the usefulness of PSA [Bok and Small, 2002]. To identify new biomarkers potentially for early diagnosis and/or therapeutics, we have characterized secreted proteins from a set of prostate cancer cell lines p69SV40T, M2205 and M12. These cell lines represent different stages of prostate cancer, namely p69SV40T as non-tumorigenic cell lines, M2205 as tumorigenic and M12 as tumorigenic and metastatic cell lines [Bae et al., 1998]. Using isotope-coded affinity tags and mass spectrometry (ICAT) [Han et al., 2001], a quantitative proteomic approach, we have identified a number of proteins that were differentially expressed in these cell lines, one of the proteins identified in this proteomic analysis was Cyr61 [Cong and Han, unpublished work]. In this report, we have characterized Cyr61 expression in prostate cancer cell lines and prostatic tissue specimens. We found that Cyr61 was up-regulated in prostate cancer cells and tumor samples. In addition, we found that the levels of Cyr61 expression closely associated with the status of the tumor suppressor gene *p53*.

MATERIALS AND METHODS

CELL CULTURE AND TISSUE SAMPLES

The cell lines RWPE-1, PWR-1E, RWPE-2, LNCaP, PC3, DU145, MCF-7, MDA-MB-435, MDA-MB-231, MDA-MB-468, A2780, HEY, SKOV3, OCC1, and HeLa were from American Tissue Collection Center. RWPE-1, PWR-1E, and RWPE-2 cells were maintained in keratinocyte-serum-free medium supplemented with 5 ng/ml human recombinant epidermal growth factor and 0.05 mg/ml bovine pituitary extract (Invitrogen, Paisley, UK); MCF-7 and HeLa cells were maintained in DMEM media (Hyclone); LNCaP, PC3, DU145, MDA-MB-435, MDA-MB-231, MDA-MB-468, A2780, HEY, SKOV3, and OCC1 cells were cultured in RPMI 1640 media (Hyclone). Media were supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Gibco). All cells were incubated at 37°C in 5% CO_2. References regarding the *p53* gene status in these cell lines will be given upon request.

Thirty-five tissue specimens from 18 BPH and 17 prostate cancer (PCa) patients were obtained after patients signed informed consent forms, and the studies were approved by the Medical Ethics Committee of Department of Pathology, General Hospital of the Second Artillery. The Human Prostate Tissue Arrays used in this study was purchased from Cybrdi Co. (Cybrdi, Frederick, MD), which consists of 80 radical prostatectomy-derived specimens from 80 different patients.

RNA ISOLATION AND RT-PCR

Total RNA was extracted from cells using Trizol (Invitrogen) according to the standard protocol. RNA samples were treated with DNase I (Ambion) and reverse transcribed by oligo(dT) primer. Synthesized cDNA was then combined in a PCR reaction with primers as follow: 5′-CAGAGGGCAGACCCTGTGAATA-3′ and 5′-GCACTGGGACCATGAAGTTGTT-3′ for Cyr61, 5′-GTTTCCGTCT-GGGCTTCTTG-3′ and 5′-CAACCTCAGGCGGCTCATAG-3′ for p53, 5′-TGCTAAGCAGTTGGTGGTGCAGGA-3′ and 5′-CGGAGTCAAC-GGATTTGGTCGTAT-3′ for GAPDH. PCR products were visualized under UV light after agarose gel electrophoresis.

WESTERN BLOTTING ANALYSIS AND IMMUNOHISTOCHEMISTRY

Cells were lysed with a cell lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). Protein concentrations were determined using the Bradford assay system. Equal amounts of protein from each sample were separated by 10% SDS–PAGE and transferred to PVDF membranes (BIO-RAD, USA). After blocking with 5% BSA (Sigma, USA) in TBS-Tween-20 (Tris 50 mM, NaCl 30 mM and 0.05% Tween-20), the membranes were incubated with a Cyr61 (1:1,000 dilution) antibody (Santa Cruz Biotechnology, Cat. No. sc-13100), a p53 (1:1,000 dilution) antibody (Cell Signaling, Cat. No. #9282) or an β-actin (1:1,000 dilution) antibody (Cell Signaling, Cat. No. #4970) for 2 h at room temperature. An alkaline phosphatase goat anti-rabbit IgG Ab (Jackson Immuno, USA) and BCIP/NBT (Sigma) was used in the chemiluminescence detection.

The immunohistochemical staining was carried out in 10% (v/v) formalin-fixed, paraffin-embedded tissue sections (5 μm thickness). All sections were dried at 60°C for 4 h. Antigen retrieval was done by steaming the slides in 10 mmol/L citrate buffer (pH 6.0) for 5 min. After antigen retrieval and H_2O_2 (3%; v/v) treatment to block endogenous peroxidase, the sections were washed with ddH_2O, then incubated with the Cyr61 antibody (1:500) in PBS at 4°C overnight. After washing, alkaline phosphatase-conjugated secondary antibody was added (1:200). Immunoreactivity was detected with the DAB staining system (Cymed, USA). After staining, the sections were counterstained with Gill's hematoxylin, dehydrated with graded alcohol and xylene, and mounted with coverslips.

PLASMIDS, siRNA, AND TRANSFECTIONS

cDNA encoded the wild-type p53 or dominant-negative p53 [Serrano et al., 1997] was cloned into pcDNA3.1 expression vector to generated pcDNA3.1-p53 or pcDNA3.1-DNp53 respectively. siRNA duplexes against human *p53* mRNA (sense 5′-CUACUUC-CUGAAAACAACG-3′) and the non-specific control siRNA duplexes (sense 5′-UUCUCCGAACGUGUCACGUTT-3′) were described in Vlastimil et al. [2006] were purchased from Shanghai Genepharma Co., Ltd (China). Transfections of either expression plasmids or siRNAs were proceeded in 60-mm dishes by lipofectamine 2000 regent (Invitrogen) following the manufacturer's instructions. For stable transfection, HeLa cells were transfected with either pcDNA3.1-DNp53 or control vector pcDNA3.1 and selected with G418 (1.5 mg/ml; Gibco).

STATISTICAL METHODS

Statistical analysis were done by the test of non-parametric correlations [DeMarzo et al., 2003] for the correlation between

levels of Cyr61 and PSA, and by Mann–Whitney test for the correlations of levels Cyr61 or PSA in BPH and prostate cancer. The correlation of Cyr61 expression with Gleason score was analyzed by Kruskal–Wallis test. Differences were considered significant when $P < 0.05$.

RESULTS

Cyr61 IS DIFFERENTIALLY EXPRESSED IN CELL LINES THAT REPRESENT DIFFERENT STAGE OF PROSTATE CANCER PROGRESSION

We tested Cyr61 expression in additional prostatic cell lines. RWPE-1 (non-tumorigenic), PWR-1E (tumorigenic), and RWPE-2 (tumorigenic and metastasis) represent different stages of prostate cancer [Bello et al., 1997]. Cell lines derived from prostate cancer LNCaP, DU145, and PC3 were also included. mRNA levels of *Cyr61* in these cell lines were assessed by RT-PCR analysis. Consistent with the proteomic results, expression levels of *Cyr61* were low in non-tumorigenic RWPE-1 cells and high in PWR-1E and RWPE-2 cells (Fig. 1a). Interestingly, we observed that mRNA level of *Cyr61* was relatively low in the androgen-dependent prostate cancer cell line LNCaP, when compared to the androgen-independent and metastatic cell lines DU145 and PC3 (Fig. 1a). It has been reported that both estrogen and androgen regulate *Cyr61* expression in breast cancer cells [Sampath et al., 2001]. This observation suggested that *Cyr61* expression may be regulated by androgen in prostate cancer cells.

The expression of Cyr61 was further examined at the protein level by Western blotting analyses. Consistent with the mRNA levels, Cyr61 protein was found to be expressed at low levels in the non-tumorigenic cell line RWPE-1 and androgen-dependent cell line LNCaP, but high in tumorigenic and metastatic cell line RWPE-2, DU145, and PC3 (Fig. 1b).

TISSUE ARRAY ANALYSIS OF Cyr61 EXPRESSION BY IMMUNOHISTOCHEMICAL STAINING

We further characterized Cyr61 expression in a commercial human prostate tissue array derived from 80 individuals. By immunohistochemical staining, we found that Cyr61 was up-regulated in prostate cancer compared to BPH. Based on visual estimation for the intensities of immunohistochemical staining, we graded the expression levels of Cyr61 as 1^+ for low level, 2^+ for medium level and 3^+ for high level respectively. Representatives of immunohistochemical staining of Cyr61 expression in BPH and prostate cancer were shown in Figure 2. Statistic analysis showed that there was a close association of levels of Cyr61 expression between BPH and prostate carcinoma ($P = 0.002$) (Table I). However, there was no remarkable tendency of high expression level of Cyr61 in more advanced prostate cancer based on Gleason Score classification ($P = 0.2$). And no correlation was found between levels of Cyr61 expression versus the patient's age. Cyr61 was detected in one of two cases of transitional cell carcinoma, but undetectable in leiomyosarcoma included in this tissue array (data no shown). Together, our results showed that Cyr61 was up-regulated in prostate cancer cells and tissues.

CORRELATION BETWEEN LEVELS OF Cyr61 IN PROSTATIC TISSUES AND LEVELS OF PSA IN SERA

PSA is the most useful biomarker in the detection of prostate cancer [Stephan et al., 2000]. We examined whether there is a correlation between Cyr61 expression in tissues and PSA concentration in serum samples from BPH and prostate cancer patients.

We have analyzed the expression of Cyr61 in prostatic tissues from 18 patients with BPH and 17 patients with confirmed prostate carcinoma by immunohistochemical staining. Based on visual estimation of the intensities of Cyr61 staining, we graded the levels of Cyr61 expression with a scale of 1^+ to 3^+ as low, medium and

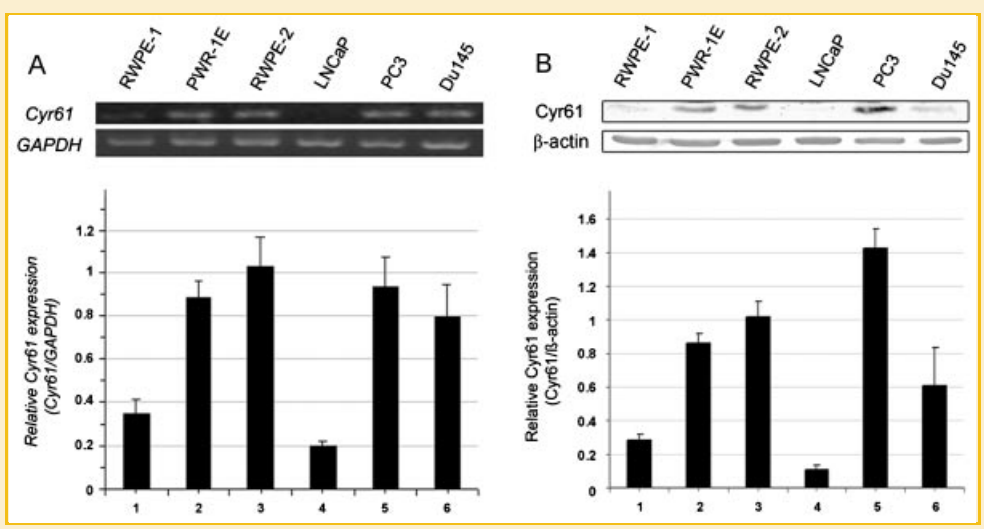

Fig. 1. Cyr61 expression in different human prostate cell lines. a: Relative mRNA levels of *Cyr61* were determined by RT-PCR analysis, a representative from three experiments with similar results (top). Relative levels of *Cyr61* were expressed as ratios of *Cyr61/GAPDH* quantified from three experiments by densitometric analysis (bottom). b: Relative Cyr61 protein levels were determined by Western blotting, a representative from three experiments with similar results (top). Relative levels of Cyr61 were expressed as ratio of Cyr61/β-actin quantified from three experiments by densitometry (bottom). *GAPDH* and β-actin were used as loading controls respectively. Columns, mean; bars, SD±.

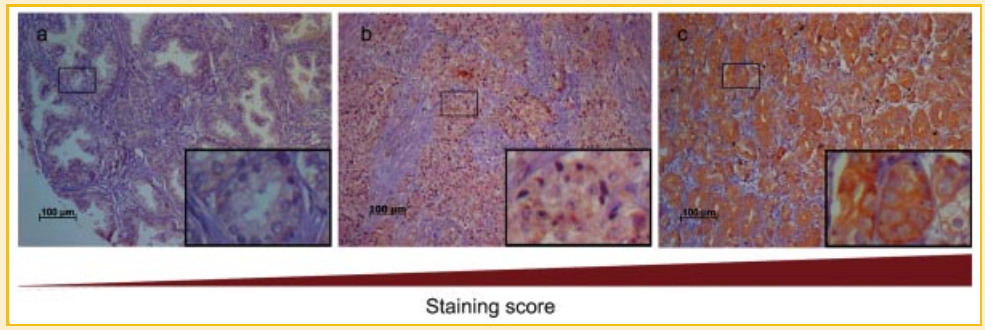

Fig. 2. Expression of Cyr61 in BPH and prostate cancer tissues. Representative immunohistochemical staining of Cyr61 in prostate tissues. a: BPH tissue in which Cyr61 expression level was low graded as 1^+. b,c: Prostate cancer tissues with high levels of Cyr61 graded as 2^+ and 3^+ respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

high. Consistent with the tissue array data, we found that expression levels of Cyr61 were low graded as 1^+, but detectable in 16/18 BPH samples, 2/18 BPH samples graded as 2^+, but majority of prostate cancer tissues examined expressed high levels of Cyr61. Two of 18 samples have low levels graded as 1^+, 5/18 samples graded as 2^+ and 11/18 samples graded as 3^+ (Fig. 3).

The concentration of PSA in patient's sera was measured in General Hospital of the Second Artillery at the time of diagnosis. As shown in Figure 3, in these 35 samples analyzed, there was a significant association of PSA concentration between BPH and prostate cancer ($P < 0.001$). PSA concentration was significantly increased in prostate cancer samples versus BPH samples. Similarly, the levels of Cyr61 expression in tissues were also significantly increased in prostate cancer samples versus BPH samples ($P < 0.001$). However, there was no correlation between levels of Cyr61 expression in tissues and PSA concentration in patient's serum ($P = 0.108$). This data suggested that Cyr61 may represent an independent biomarker for prostate cancer detection.

LEVELS OF Cyr61 EXPRESSION WAS ASSOCIATED WITH STATUS OF THE p53 GENE

As in other human cancers, mutations of p53 tumor suppressor gene are the most frequent genetic alteration in prostate cancer and can be detected in up to 94% of cases [Heidenberg et al., 1996]. Recent evidences suggest that p53 mutations might be present at earlier stage of the disease [Downing et al., 2001]. It has been shown that Cyr61 suppressed the growth of non-small-cell lung cancer cell lines by induced expression of p53 through a mechanism involving the integrin/β-catenin pathway [Tong et al., 2004], suggesting a functional interaction between Cyr61 and p53. Therefore, we compared the expression levels of Cyr61 with status of the tumor suppressor gene *p53* in different cancer cell lines by Western blotting analysis. As shown in Figure 4, low levels of Cyr61 were found in cell lines with wild-type *p53*, whereas high levels of Cyr61 found in cell lines with mutant *p53* or null *p53*, with only one exception for HEY. To gain more comprehensive information of the relationship between Cyr61 expression and the *p53* gene status, we have reviewed data reported in the published literatures [Tong et al., 2004; Xie et al., 2001, 2004b] and in the cell lines used in this report, and compared the levels of Cyr61 expression with the *p53* gene status, which is known in those cell lines. In a total of 32 cell lines analyzed, We found that the levels of Cyr61 expression is closely associated with the *p53* gene status, low levels of Cyr61 found in cell lines with wild-type *p53* (10/32), whereas high levels of Cyr61 found in cell lines with mutant *p53* or null *p53* (18/32), with only four exceptions for the cell lines HEY, H526, U87, and BT-20 (Table II), indicating a functional link between Cyr61 and p53 in cancer.

To confirm that Cyr61 expression is associated with *p53* gene status, we transiently transfected a dominant-negative *p53* expression construct or a small interference RNA against endogenous *p53* [Vlastimil et al., 2006], into a *p53* wild-type HeLa cell line. Levels of *Cyr61* mRNAs and *p53* mRNAs (including the wild-type and dominant negative *p53*) were assayed by RT-PCR. As shown in Figure 5a, over-expression of the dominant negative *p53*, or down-regulated expression of endogenous wild-type *p53* by *p53* siRNA,

TABLE I. Differential Expression of Cyr61 in BPH and Prostate Cancer

Groups		n	Age, mean (range)	Staining 1^+, n (%)	Staining 2^+, n (%)	Staining 3^+, n (%)	*P*
BPH		9	50.3 (21–77)	7 (77.8%)	2 (22.2%)	0	0.002
Prostate cancer		67	69.1 (40–87)	17 (25.4%)	27 (40.3%)	23 (34.4%)	
Tumor grade (Gleason score)	1	10	67.1 (56–76)	3 (30.0%)	6 (60.0%)	1 (10.0%)	0.200
	2	10	74.3 (64–85)	2 (20.0%)	3 (30.0%)	5 (50.0%)	
	3	17	67.9 (51–82)	3 (17.6%)	6 (35.3%)	8 (47.1%)	
	4	18	68.0 (40–81)	4 (22.2%)	7 (38.9%)	7 (38.9%)	
	5	12	69.6 (55–87)	5 (41.7%)	5 (41.7%)	2 (16.7%)	

Human prostate tissue array analysis of Cyr61 expression. Immunohistochemical analysis of Cyr61 expression in a human prostate tissue array. Levels of Cyr61 expression were assigned 1^+ as low, 2^+ as medium and 3^+ as high expression, respectively.

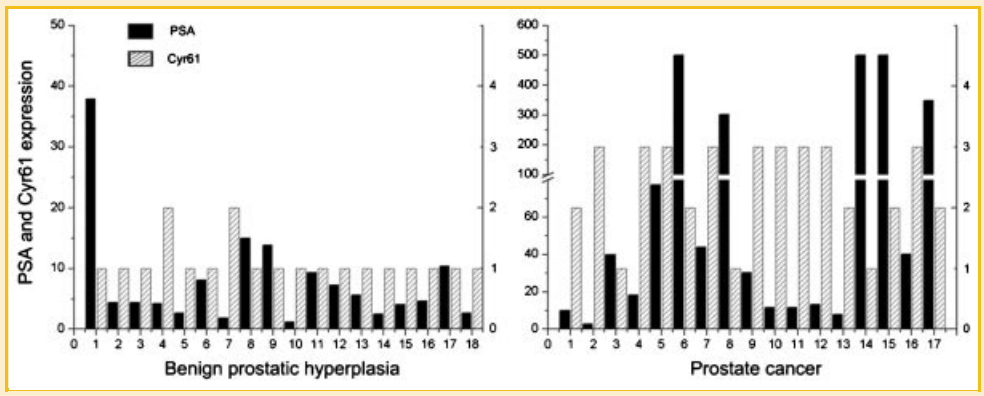

Fig. 3. Expression level of Cyr61 and PSA in BPH and prostate cancer patients. Relative Cyr61 expression determined by immunohistochemical staining using Cyr61 antibody, expression levels were assigned based on the immunohistochemical staining intensities as follow: 0 as undetectable, 1^+ as low, 2^+ as medium, 3^+ as high expression, respectively. Concentration of PSA (ng/ml) in patient's serum was measured in the General Hospital of the Second Artillery at time of diagnosis. Left *y*-axis represents concentration (ng/ml) of PSA and right *y*-axis represents levels of Cyr61 expression. Eighteen BPH and 17 prostate cancers were analyzed (*x*-axis).

up-regulated *Cyr61* mRNA expression in a dose-dependent manner. Similarly, levels of *Cyr61* mRNA as well as levels of Cyr61 protein were significantly increased in HeLa cells stably transfected by the dominant negative *p53* expression construct (Fig. 5b). Moreover, when we transiently transfected the wild-type *p53* expression construct into two *p53* null cell lines, prostate cancer cell line DU145 and breast cancer cell line MDA-MB-468, transiently over-expressed wild-type p53 resulted in down-regulated *Cyr61* mRNA expression (Fig. 5c). Together, these data indicated that wild-type and dominant-negative p53 differentially regulated Cyr61 expression, suggesting a functional interaction between Cyr61 and p53 in cancers.

DISCUSSIONS

Early stage prostate cancer confined to the prostate gland can be cured by surgery. In contrast, advanced prostate cancer is invasive and it is mainly treated in clinical by androgen ablation therapy. However, cancer cells occasionally escape this treatment and behave as androgen independent prostate tumor and that is currently incurable disease. The early detection of cancer is crucial

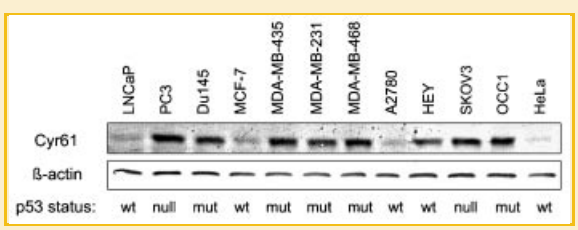

Fig. 4. Association of Cyr61 expression and the status of *p53*. Expression of Cyr61 in different cancer cell lines were examined by Western blotting analysis using the Cyr61 antibody, β-actin was used as loading controls. Representative of the three experiments with similar results.

TABLE II. Levels of Cyr61 Expression Associated With Status of the Tumor Suppressor Gene *p53*

Cell lines	Cyr61 levels	*p53* status
Prostate cancer [data in this report]		
LNCaP	Low	WT
PC3	High	Null
Du145	High	Mutant
Breast cancer [Xie et al., 2001 and data in this report]		
MCF-7	Low	WT
ZR75-1	Low	WT
BT-20	Low	Mutant
BT-474	High	Mutant
T47D	High	Mutant
MDA-MB-157	High	Null
MDA-MB-436	High	Mutant
MDA-MB-435	High	Mutant
MDA-MB-468	High	Null
MDA-MB-231	High	Mutant
Ovarian cancer [data in this report]		
A2780	Low	WT
HEY	High	WT
SKOV3	High	Null
OCC1	High	Mutant
Cervix cancer [data in this report]		
HeLa	Low	WT
Lung cancer [Tong et al., 2004]		
H446	Low	WT
H460	Low	WT
H520	Low	WT
H187	Low	WT
H526	Low	Mutant
H1299	High	Null
H125	High	Mutant
H157	High	Mutant
Gliomas [Xie et al., 2004b]		
U343	Low	WT
U87	High	WT
U138	High	Mutant
U118	High	Mutant
U373	High	Mutant
T98G	High	Mutant

Levels of Cyr61 expression are correlated with *p53* gene status in different cancer cell lines. The levels of Cyr61 expression in different cancer cell lines from the published literatures and cell lines used in this study were summarized and compared with the status of the *p53* gene in each cell lines.

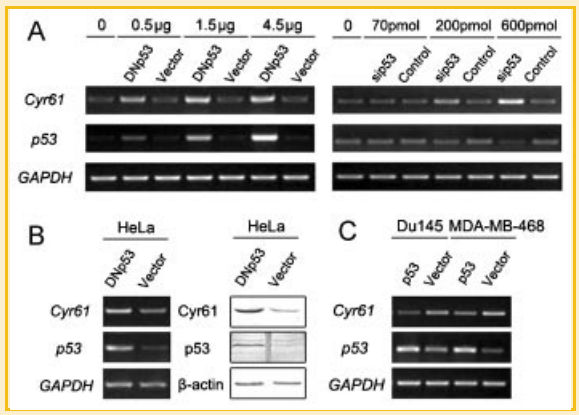

Fig. 5. Wild-type and mutant p53 differentially regulated Cyr61 expression. a: Over-expression the dominant-negative p53 up-regulated Cyr61 expression in HeLa cells in a dose-dependent manner. HeLa cells were transiently transfected with indicated amount of the dominant-negative *p53* expression construct or *p53* siRNA. mRNA levels of *Cyr61* and *p53* were assessed by RT-PCR after 48 h post-transfection. b: Cyr61 was up-regulated in HeLa cells stably transfected with the dominant-negative *p53* construct. HeLa cells were stably transfected with dominant-negative *p53* construct. Levels of mRNAs and proteins of Cyr61 and p53 were analyzed by RT-PCR and Western blot. c: Over-expression of wild-type p53 down-regulated Cyr61 expression in DU145 and MDA-MB-468 cells. DU145 and MDA-MB-468 cells were transiently transfected with wild-type *p53* construct, mRNA levels of *Cyr61* and *p53* were assessed by RT-PCR.

for its ultimate control and prevention. PSA has been used clinically as a diagnosis biomarker. However, sensitivity and specificity of prostate cancer detection needs to be further improved.

Cyr61 was originally reported to be down-regulated in prostate cancer [Pilarsky et al., 1998], but more recent studies indicate that Cyr61 is up-regulated in BPH and it promotes prostatic stromal and epithelial cell proliferation [Sakamoto et al., 2003, 2004a,b]. The reason for these conflicting reports is unknown. Expression of Cyr61 in prostate tumors has not been examined and its role in prostate cancer progression remains elusive.

In this study, we found that Cyr61 was up-regulated in prostate cancer cells and tumor samples. There was a close association of Cyr61 expression between BPH and prostate cancer. However, but there was no correlation between the expression levels of Cyr61 and that of PSA, and no correlation was found between Cyr61 expression levels and prostate cancer progression based on Gleason Score Classification. These suggest that alterations in Cyr61 expression might be present at earlier stage of prostate cancer. Therefore, Cyr61 could be considered as an independent biomarker complementary to PSA in early prostate cancer detection, where patients exhibiting low preoperative PSA or low Gleason had a better prognosis.

We observed that levels of Cyr61 expression were low in androgen dependent cell line LNCaP but high in androgen independent cell lines DU145 and PC3. It has been reported that both estrogen and androgen regulate Cyr61 expression in breast cancer cells [Sampath et al., 2001]. These observations suggest that Cyr61 expression may be regulated by androgen in prostate cancer cells and is a potential therapeutic target for prostate cancer.

Prostate cancer progression involves sequential genetic changes, several chromosome alterations as well as the inactivation of tumors suppressors such as RB and p53 have been frequently detected in prostate cancer. Interestingly, we found that the levels of Cyr61 expression is closely correlated with the *p53* gene status, low levels of Cyr61 associated with wild-type *p53*, whereas high levels of Cyr61 associated with mutant *p53* or null *p53*, with only few exceptions (Table II, Fig. 4).

Importantly, we experimentally showed that transient and stable expression of the dominant-negative p53, or down-regulation of endogenous wild-type p53 resulted in up-regulated of Cyr61 in HeLa cells. In addition, we showed that transiently over-expression of wild-type p53 in two *p53* null cancer cell lines DU145 and MDA-MB-468 suppressed Cyr61 expression. And, inhibition of p53 function by either transiently or stably transfection of the dominant-negative *p53* expression construct has similar effect on Cyr61 expression in MCF-7 and LNCaP cell lines (data not shown).

It has been shown that Cyr61 suppressed the growth of non-small-cell lung cancer cell lines by induction of p53 expression through a mechanism involving the integrin/β-catenin pathway [Tong et al., 2004]. We found that over-expression of Cyr61 by transfection of *Cyr61* expression construct into LNCaP, MCF-7 and HeLa cells, which express low level of Cyr61 and wild-type *p53*, or suppression of Cyr61 expression by *Cyr61* siRNA in DU145, MDA-MB-231 and OCC1 cells, which express high level of Cyr61 and mutant *p53*, has no effect on p53 expression (data not shown). More recent study indicates that Cyr61 can either induce or suppress apoptosis in p53-dependent and cell type-specific manner [Todorovicç et al., 2005]. Therefore, functional interaction between Cyr61 and p53 may be cell type-specific. Although, mechanisms of regulation between Cyr61 and p53 in different cellular context remain to be elucidated, functional link between Cyr61 and p53 may exit in cancers. Given the critical roles of p53 in tumorigenesis, understanding the significance of the association between Cyr61 expression and the *p53* gene status, and the mechanism of p53 regulated Cyr61 expression would provide insights into the function of Cyr61 in prostate cancer biology.

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