Interaction of cyclophilin A with a novel binding protein, SR-25, and characterization of their expression pattern in Chinese hepatocellular carcinoma patients

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Abstract. Cyclophilin (Cyp) A has been reported to be overexpressed in the majority of cancer cells, including hepatocellular carcinoma (HCC). However, the biological functions of CypA in HCC are far from being understood. To determine the biological functions of CypA in HCC, the present study screened human fetal liver complementary DNA for proteins interacting with CypA using the yeast two-hybrid system. A nuclear protein, serine/arginine-rich (SR)-25, was isolated as a novel CypA-binding protein that is distinct from those previously described in the literature. Binding assays and co-immunoprecipitation confirmed the physical association between CypA and SR-25. The present study demonstrated that CypA may interact with SR-25 through its peptidyl-prolyl isomerase domain. In addition, CypA may induce the expression of SR-25 in Hep3B cells. The messenger RNA levels of CypA and SR-25 in HCC indicated that there was a significant correlation between the expression of CypA and the expression of SR-25 in HCC. It can be speculated that the interaction between CypA and SR-25 proteins may be involved in potential carcinogenic functions of CypA in HCC. Further studies will focus on elucidating in detail the molecular mechanisms of the interaction between CypA and SR-25.

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Introduction

Cyclophilins (Cyps) are a group of cellular proteins known as immunophilins, which display the enzymatic activity of peptidyl-prolyl isomerases (PPIases) (1). These enzymes catalyze the cis to trans conversion of proline-containing peptides (1,2). CypA is a key member of the Cyp family, and was first shown to mediate the immunosuppressive function of cyclosporine (Cs) A through the formation of a CsA-CypA complex (3). As a multifunctional chaperone, CypA has been demonstrated to participate in a range of cellular functions, including protein folding, trafficking, immunomodulation and cell signaling (4). Previously, CypA was shown to participate in numerous important processes, including exacerbation of oxidative stress (5), inflammation (5), pathogenesis of vascular diseases (6), human immunodeficiency virus infection (7) and rheumatoid arthritis (8). Furthermore, overexpression of CypA often correlates with severity of cancers, including pancreatic and hepatic cancer, suggesting its role in tumor malignancies (3).

Human hepatocellular carcinoma (HCC) is one of the most common malignances in the world, with a particular high prevalence in Asian and African countries (9). HCC is the third-leading cause of cancer-associated mortalities, and the survival rate is 30-40% at 5 years post-surgery (10). Infection with hepatitis B and C viruses is considered to account for >80% of primary liver cancers (11,12). As an essential host factor, CypA is critical for virus replication, and may facilitate hepatotropic viral infections (13). In addition, CypA was initially noticed to be upregulated in HCC, and may promote HCC metastasis through the upregulation of matrix metalloproteinase (MMP)3 and MMP9 (14). To date, CypA has been considered as a potential therapeutic target for molecular cancer therapy, due to its important role in tumor formation and metastasis (3). However, the biological functions of CypA in HCC are far from being understood.

The present study screened human fetal liver complementary DNA (cDNA) for proteins interacting with CypA using the yeast two-hybrid system. A nuclear protein, serine/arginine-rich (SR)-25, was isolated as a novel protein that is distinct from the CypA-binding proteins previously described in the literature. Binding assays and co-immunoprecipitation were used to

confirm the physical association between CypA and SR-25. Furthermore, the messenger RNA (mRNA) levels of SR-25 and CypA in 24 HCC cases were also evaluated in the present study.

Materials and methods

Isolation of interacting proteins. The Matchmaker LexA Two-Hybrid System (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to isolate the CypA-interacting proteins following the manufacturer's protocol. *Saccharomyces cerevisiae* EGY48 was transformed first with the p8op-LacZ reporter plasmid, and then with pLexA-CypA. A single colony grown in selective synthetic defined (SD) medium lacking uracil and histidine (Ura^-His^-) was transformed with the pB42AD activation domain plasmid (Clontech, Inc.) of the human fetal liver cDNA library provided in the Matchmaker LexA Two-Hybrid System. Interacting plasmids were selected using SD/galactose/raffinose/Ura^-His^-leucine$^-$ medium and verified by sequencing.

Plasmid construction. For expression of CypA in the *Escherichia coli* strain BL21 (Novagen, Inc., Madison, WI, USA), human CypA cDNA (GenBank accession number NM_021130) was inserted in frame into the pGEX6P-1 vector (GE Healthcare Life Sciences, Chalfont, UK). The CypA PPIase mutation CypAm (R55A and F60A) was also constructed as previously described (15).

To investigate their subcellular localization, human CypA and SR-25 (GenBank accession number NM_016638) cDNAs were introduced into the pCMV-Myc (Clontech Laboratories, Inc.) and pCMV-Flag (Clontech Laboratories, Inc.) vectors, respectively.

Cell culture and transfection. Hep3B cells (American Type Culture Collection, Manassas, VA, USA) were grown in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal calf serum (Thermo Fisher Scientific, Inc.). Cells ($3.5x10^5$) were seeded in 60-mm dishes. Upon overnight growth, cells were 80% confluent, and were transfected with 3 μg of plasmid constructs using Lipofectamine Reagent (Thermo Fisher Scientific, Inc.) in serum-free medium. After 5 h of incubation, the medium was replaced with fresh complete medium, and cells were cultured for an additional 48 h prior to collection.

Glutathione S-transferase (GST)-fusion protein pull-down experiments. Hep3B cells that expressed Flag-SR-25 were harvested and lysed in 500 μl lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1 mM PMSF and 10 μg/ml each aprotinin and leupeptin). Cell lysates were centrifuged at 10,000 x g for 10 min at 4 °C. The expression and purification of GST fusion proteins was conducted following the protocol provided by the manufacturer of Glutathione Sepharose 4B (GE Healthcare Life Sciences). Purified GST, GST-CypA or GST-CypAm (R55A and F60A) proteins were covalently attached to the 50% slurry of Glutathione Sepharose 4B beads, and then incubated with the whole-cell lysates of cells expressing Flag-SR-25 at 4˚C for 3 h. The beads were washed three times with lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1 mM PMSF and 10 μg/ml each aprotinin and leupeptin), and the bound proteins were analyzed by western blotting using an anti-Flag monoclonal antibody (mAb; 1:1,000; F1804; Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) and an anti-GST mAb (1:1,000; sc-33613; Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

Immunoprecipitation. Cells lysates were pre-clarified with Protein A/G Plus Agarose (Thermo Fisher Scientific, Inc.) by rotating at 4˚C for 30 min. Upon separation from the beads by centrifugation (at 1,000 x g 2 min and 4˚C), the lysates were then immunoprecipitated with ANTI-FLAG M2 Affinity Agarose Gel (Sigma-Aldrich) for 3 h at 4˚C. The beads were washed four times with the aforementioned cell lysis buffer and finally analyzed by western blotting using anti-Flag mAb and anti-GST mAb.

Western blotting. Samples were separated by 10% SDS-PAGE, followed by transfer to polyvinylidene difluoride membranes. Upon blocking with PBS containing 5% bovine serum albumin (BSA; Sigma-Aldrich) and 0.1% Tween 20, the membrane was incubated with appropriate primary antibodies at room temperature for 2 h, followed by incubation with a peroxidase-conjugated goat anti-rabbit IgG or peroxidase-conjugated goat anti-mouse IgG (both 1:5,000; ZB-2301; ZB-2305; ZSGB-BIO, Beijing, China) at room temperature for 1 h. The signals were detected using Western Blotting Luminol Reagent (Santa Cruz Biotechnology, Inc.). The primary antibodies were anti-Flag mAb (1:1,000; F1804; Sigma-Aldrich), anti-GST antibody (1:1,000; sc-33613; Santa Cruz Biotechnology, Inc.) and anti-Myc antibody (1:1,000; A7470; Sigma-Aldrich).

Immunofluorescence analysis. Hep3B cells grown on coverslips were fixed in 4% paraformaldehyde for 10 min and washed once with TBS. Fixed cells were permeabilized with 0.2% Triton X-100 for 5 min, washed three times with TBS and incubated for 5 min in 0.1% sodium borohydride (freshly prepared in TBS) to quench endogenous fluorescence. Upon blocking with blocking buffer [1% horse serum (Thermo Fisher Scientific, Inc.) 1% BSA, 0.02% NaN_3 and 1X PBS] for 1 h, the cells were incubated with primary rabbit anti-Myc mAb (1:500; A7470; Sigma-Aldrich) and mouse anti-Flag mAb (1:500; F1804; Sigma Aldrich) at 4˚C overnight. The coverslips were then rinsed three times in PBS and reacted with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (green; 1:200; ZF0312; ZSGB-BIO) and rhodamine-conjugated goat anti-rabbit IgG (red; 1:200; ZF0316; ZSGB-BIO) secondary antibodies in the dark for 1 h. Subsequently, cells were counterstained with 1 μg/μl DAPI (Shanghai Yeasen Biotechnology Co., Ltd., Shanghai, China) at 37˚C for 20 min and mounted on glass slides prior to visualization. Images were recorded using a DC500 camera (Leica Microsystems, Inc., Buffalo Grove, IL, USA) on a microscope equipped with DMRA2 fluorescence optics (Leica Microsystems, Inc.).

Tumor samples. A total of 24 pairs of primary HCC tissue samples and adjacent tumor-free tissue samples were obtained at Yantai Yuhuangding Hospital (Yantai, China). The samples were obtained from patients during cytoreductive

Table I. Interaction of CypA and SR-25 in the yeast two-hybrid system.

Experiments	P_{ADH1}-BD fusion	P_{GAL1}-AD fusion	SD minimal medium	Colony growth/color
Interaction test	PlexA-CypA	pB42-SR-25	SD-4[a]	Blue
Negative control	PlexA-CypA	pB42	SD-4[a]	No growth
Negative control	PlexA-CypJ	pB42	SD-4[a]	No growth
Negative control	PlexA	pB42-SR-25	SD-3[b]	White
Negative control	PlexA	pB42	SD-3[b]	White
Negative control	PlexA	-	SD-2[c]	White
Negative control	-	pB42	SD-2[c]	White

[a]SD-4, SD/Gal/Raf/His^-/Leu^-/Trp^-/Ura^-. [b]SD-3, SD/Gal/Raf/His^-/Trp^-/Ura^-. [c]SD-2, SD/Gal/Raf/Trp^-/Ura^-. All media contained X-gal. Blue staining indicated positive interaction. White staining indicated negative interaction." Cyp, cyclophilin; SR, serine/arginine-rich; SD, synthetic defined; Plex, plexin; Gal, galactose; Raf, raffinose; His, histidine; Leu, leucine; Trp, tryptophan; Ura, uracil; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; PADH1-BD fusion, PADH1-BD fusion vector; PGAL1-AD fusion, PGAL1-AD fusion vector.

surgery between January 2013 and May 2015. No patient was administered radiotherapy or chemotherapy prior to surgery. All experimental procedures were approved by the Ethics Committee of Yantai Yuhuangding Hospital. Informed consent was obtained from all patients prior to the procedure.

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA was isolated from cultured cells or tissue samples by a single-step isolation method using TRIzol reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA (2 μg) was reverse transcribed with ReverTra Ace-α-® (Toyobo Co., Ltd., Osaka, Japan). PCR was performed under the following conditions: A 5-min initial denaturation step at 94˚C, followed by 29 cycles of denaturation at 94˚C for 30 sec, annealing at different temperatures for 30 sec and extension at 72˚C for 30 sec, and a final extension step at 72˚C for 10 min. The forward and reverse primers were selected to span several introns to avoid genomic DNA amplification. Amplimer contamination was controlled with a complete PCR reaction mixture without cDNA. The primer sequences were as follows: CypA forward (F), 5'-TACGGGTCCTGGCATCTT-3' and reverse (R), 5'-CAGTCAGCAATGGTGATCTTCT-3'; SR-25 F, 5'-CCTCCTCTTCTTCCAGTTCTTC-3' and R, 5'-ATTCGGGACTTCTGCTCATC-3'; β-macroglobulin (MG) F, 5'-ATGAGTATGCCTGCCGTGTGAAC-3' and R, 5'-TGTGGAGCAACCTGCTCAGATAC-3'; and GAPDH F, 5'-TGTGTCCGTCGTGGATCTGA-3' and R, 5'-TTGCTGTTGAAGTCGCAGGAG-3'.

The semi-quantitative RT-PCR results were scanned with Syngene G:BOX EF2 (Syngene, Frederick, MD, USA) and analyzed using GeneTools image analysis software version 4.02 (Syngene), according to Li *et al* (16). The CypA and SR-25 mRNA level in cancer and normal tissues was calculated using the dosage ratio (DR) of the ethidium bromide intensity of the SR-25/β-MG and CypA/β-MG bands in a 1.5% agarose gel.

Statistical analysis. The correlation between the scoring of SR-25 and CypA expression levels was analyzed by the exact permutation test for Spearman correlation coefficient. $P<0.05$ was considered to indicate a statistical significant difference. All statistical analyses were performed using SPSS version 19 (IBM SPSS, Armonk, NY, USA).

Results

Screening of proteins that interact with CypA. To identify the proteins that interact with CypA, a total of $2x10^7$ transformants were screened from a human fetal liver cDNA library, and the initial screen revealed 43 cDNA clones. By restriction mapping, nine of these candidate clones with the same length of 1.2 kb were selected for further study. DNA sequence analysis revealed that this set of cDNA molecules encoded the human SR-25 protein. The binding specificity between CypA and SR-25 was analyzed in yeast using pLexA-CypA and pB42-SR25 combined with different control constructs, as shown in Table I. All yeast transformants in negative control experiments either did not grow or became blue on appropriate SD minimal medium, indicating a specific interaction between CypA and SR-25 in yeast.

Interaction of SR-25 with CypA in vitro. Using a GST-CypA fusion protein, *in vitro* GST pull-down assays were performed. The results indicated that Flag-SR-25 protein associated with GST-CypA fusion protein (Fig. 1A, lane 4), but not with control GST proteins (Fig. 1A, lane 2). In addition, the PPIase mutation of CypA (CypAm, R55A and F60A) affected the interaction of CypA with SR-25, as no band was detected when Flag-SR-25 was incubated with CypAm (Fig. 1A, lane 3).

Furthermore, the interaction of SR-25 with CypA in Hep3B cells was also detected by immunoprecipitation experiments. The results revealed that, when Flag-SR-25 was immunoprecipitated, Myc-CypA co-precipitated with SR-25 (Fig. 1B, lane 6).

CypA and SR-25 co-localize at the cell nucleus. Immunofluorescence microscopic examination revealed that SR-25 and CypA co-localized mainly in the nuclei of co-transfected Hep3B cells in a diffused pattern (Fig. 2), which supported the hypothesis that SR-25 and CypA physically interact with each other.

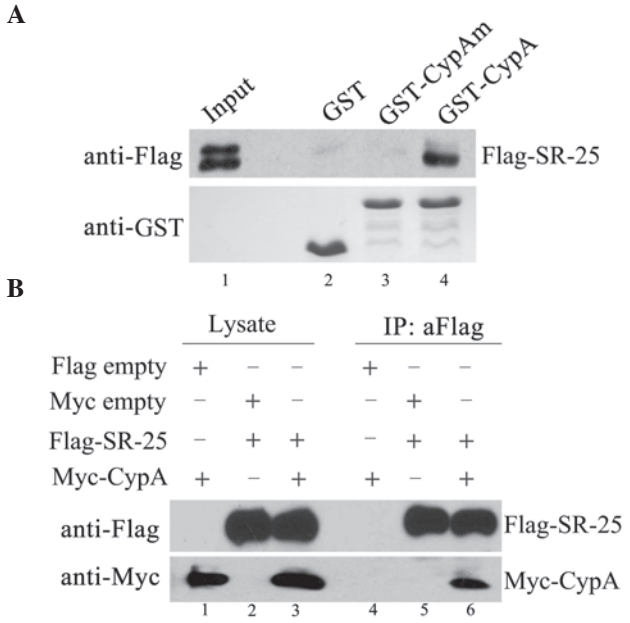

Figure 1. Interaction of SR-25 with CypA *in vitro*. (A) Interaction of SR-25 with CypA by GST pull-down assay. Immobilized 25 μg of GST (lane 2), GST-CypA (lane 3) or GST-CypAm (lane 4) proteins were incubated with extracts prepared from Hep3B cells expressing Flag-SR-25. Bound Flag-SR-25 was detected by immunoblotting with anti-Flag mAb or anti-GST mAb as control. Lysates from cells were loaded as a standard (lane 1). (B) Interaction of CypA with SR-25 by co-immunoprecipitation. Hep3B cells were transiently transfected with the indicated combination of plasmids. Cell lysates were immunoprecipitated with ANTI-FLAG M2 Affinity Agarose Gel and then subjected to western blotting with anti-Myc or anti-Flag mAbs. GST, Glutathione S-transferase; Cyp, cyclophilin; CypAm; CypA mutation (R55A and F60A); SR, serine/arginine-rich; IP, immunoprecipitation; mAb, monoclonal antibody; aflag, anti-Flag.

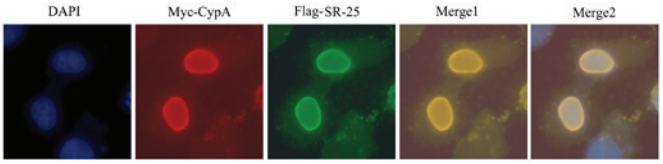

Figure 2. Co-localization of CypA and SR-25 in Hep3B cells. Hep3B cells were cultured on coverslips, transfected with the corresponding expression vectors, and analyzed by immunofluorescence with antibodies against Flag (green) and Myc (red). DNA was counterstained with DAPI (blue). Magnification, x400. Merge 1, merge of channels 2 and 3. Merge 2, merge of channels 1, 2 and 3. Cyp, cyclophilin; SR, serine/arginine-rich; mAb, monoclonal antibody.

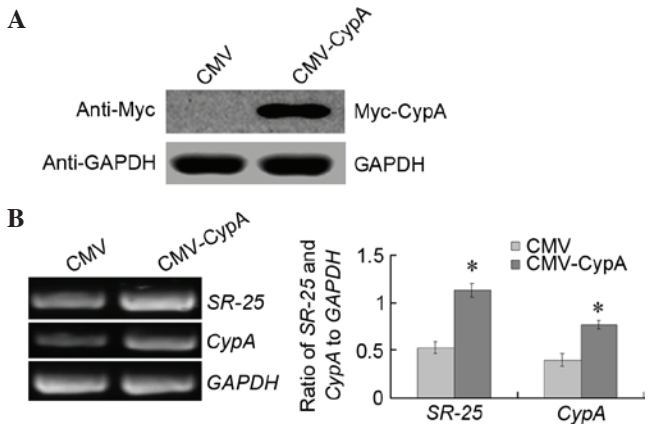

Figure 3. Expression of SR-25 following CypA overexpression. (A) Western blotting revealed elevated expression of CypA in transfected Hep3B cells with GAPDH as an internal control. (B) The expression of SR-25 and CypA was detected by reverse transcription-polymerase chain reaction upon CypA transfection. The expression of GAPDH was used as an internal control. The quantification of SR-25 and CypA messenger RNA expression is shown in the right panel, in which the relative expression represented the optical density of each sample band compared with the optical density of the GAPDH control band. Results are expressed as the mean ± standard deviation of three independent experiments. *P<0.05, Student's t-test. Cyp, cyclophilin; SR, serine/arginine-rich; CMV, cytomegalovirus.

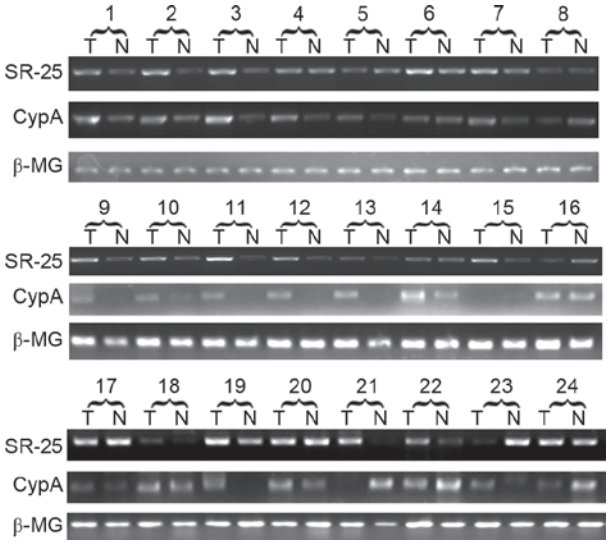

Figure 4. Semi-quantitative reverse transcription-polymerase chain reaction analysis of human SR-25 and CypA expression in 24 hepatocellular carcinoma cases. T, tumor tissue; N, adjacent normal tissue; Cyp, cyclophilin; SR, serine/arginine-rich; MG, macroglobulin.

SR-25 is upregulated by CypA overexpression in vitro. To detect if the expression of SR-25 was enhanced by CypA overexpression, CypA was overexpressed in Hep3B cells transiently transfected with the pCMV-CypA-Myc plasmid for 36 h. Overexpression of CypA was confirmed at the protein and mRNA level (Fig. 3). Semi-quantitative RT-PCR analysis was conducted to determine the expression of SR-25. The results indicated that the expression of SR-25 was upregulated by >2-fold upon transient transfection of the cells with the CypA-expression vector (Fig. 3B, right panel) (P<0.001).

CypA and SR-25 exhibit elevated expression in HCC tissues. The expression of CypA and SR-25 in 24 pairs of HCC/adjacent non-cancerous tissues was determined and compared at the mRNA level via semi-quantitative RT-PCR. The results revealed that CypA and SR-25 shared a similar expression pattern in HCC (Fig. 4). The DR of SR-25 and CypA in each case was calculated, and the results are presented in Table II. SR-25 was upregulated in 33.3% of the 24 HCC cases (8/24), while CypA was upregulated in 50% of cases (12/24).

Statistical analysis demonstrated that there was a significant positive correlation between SR-25 and CypA (Fig. 5). The Spearman correlation coefficient between SR-25 and CypA was 0.4747 (P<0.05).

Table II. Characteristics of HCC patients.

N	Age (years)	Gender	Family history	AFP (ng/ml)	HbsAg (+/-)	Tumor (n)	Size	HCC Edmondson grade	Fibrous capsule (+/-)	Cancer embolus (+/-)	SR-25[a]	CypA[a]
1	34	M	+	-	+	Sol	S	II	+	-	→	↑
2	66	M	+	-	+	Sol	S	II-III	+	-	→	→
3	71	M	-	-	+	Sol	S	I	-	-	↑	↑
4	55	F	-	-	+	Sol	S	I	-	-	→	↑
5	63	M	+	-	+	Sol	S	II-III	+	-	→	↑
6	49	M	-	+	+	Sol	L	III	-	-	→	→
7	52	M	-	-	+	Sol	S	III	-	-	↑	↑
8	61	F	+	+	+	Sol	S	II	+	-	→	→
9	58	M	-	+	+	Sol	S	II-III	-	-	→	↑
10	57	M	+	+	+	Sol	L	III	+	-	→	↑
11	62	M	+	+	+	Sol	L	III	-	-	↑	↑
12	54	F	-	+	+	Sol	S	II	+	-	↑	↑
13	50	M	+	+	+	Sol	S	III	+	-	→	↑
14	48	M	+	-	+	Sol	S	III	-	-	→	↑
15	38	F	+	-	+	Sol	L	II	+	-	↑	→
16	35	M	-	-	+	Sol	S	II	+	-	→	→
17	45	M	+	+	+	Sol	S	III	+	-	→	→
18	53	M	-	-	+	Sol	L	II-III	+	-	↑	→
19	40	M	-	+	+	Sol	S	II	+	-	↑	↑
20	65	F	-	-	+	Sol	S	II	+	-	→	→
21	51	M	-	+	+	Sol	S	III	+	-	↑	↓
22	82	M	-	+	+	Sol	L	III	+	-	→	↓
23	50	M	-	+	+	Sol	L	III	+	+	↑	→
24	53	M	+	-	+	Sol	S	II	+	-	→	↓

[a]DR = TR/NR; TR = RT-PCR products density of CypA or SR-25/RT-PCR products density of β-MG in tumor tissue; NR = RT-PCR products density of SR-25 or CypA/RT-PCR products density of β-MG in adjacent tumor-free tissue; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein (+, ≥40 mg/l; -, <40 mg/l); Sol, solitary; S, small (diameter, <5 cm); L, large (diameter, ≥5 cm); ↑, upregulated expression (DR ≥1.5); ↓, downregulated expression (DR <0.67); →, no significant expression change (0.67<DR<1.50); HCC, hepatocellular carcinoma; F, female; M, male; Cyp, cyclophilin; SR, serine/arginine-rich; DR, dosage ratio; RT-PCR, reverse transcription-polymerase chain reaction; MG, macroglobulin; N, number; TR, tumor tissue density ratio; NR, adjacent normal tissue density ratio.

Discussion

Previous studies demonstrated that CypA is one of the most abundant proteins in the cytoplasm, which accounts for 0.1% of total cytosolic proteins (17,18). However, several previous immunolocalization studies reported that CypA may also be present in the nucleus, and that CypA was implicated in several cellular processes through interacting with known nuclear proteins (19,20). Using the yeast two-hybrid system, the present study isolated a novel CypA-binding nuclear protein, SR-25. SR-25 is a novel member of a highly conserved family of splicing factors, the SR proteins (21,22). SR proteins are major modulators of alternative splicing, and usually contain a SR domain that is required for protein-protein interactions during splicing (23-25). It has been suggested that SR proteins may be important in the expression of specific disease phenotypes via alternative splicing of disease-causing genes or mutation-triggered alternative splicing events (26-28). Based on the presence of

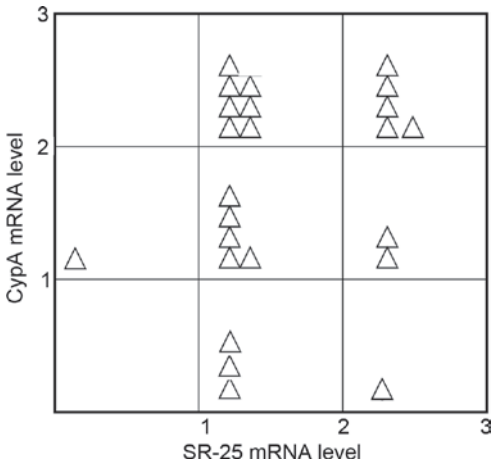

Figure 5. Correlation of SR-25 mRNA level with CypA on mRNA level. 1, DR<0.67; 2, 0.67≤DR<1.50; 3, DR≥1.50. DR = TR/NR; TR = RT-PCR product density of CypA or SR-25/RT-PCR product density of β-MG in tumor tissue; NR = RT-PCR product density of CypA or SR-25/RT-PCR product density of β-MG in adjacent tumor-free tissue. Cyp, cyclophilin; SR, serine/arginine-rich; DR, dosage ratio; RT-PCR, reverse transcription-polymerase chain reaction; TR, tumor tissue density ratio; NR, adjacent normal tissue density ratio.

numerous nuclear localizing signals and their similarity to RNA splicing proteins, SR-25 was considered to contribute to RNA splicing (29). In the present study, both the binding assay and the co-immunoprecipitation assay confirmed the physical association between CypA and SR-25. Furthermore, our study revealed that the expression of SR-25 may be induced by CypA. It may be speculated that the interaction between CypA and SR-25 proteins may be involved in potential carcinogenic functions of CypA in HCC.

Various PPIases have been reported to interact with transcription factors and to affect their activity (30,31). It can be speculated that the PPIase activity may be involved in the interaction between CypA and SR-25. SR proteins are phosphoproteins, and their phosphorylation status can affect their ability to interact with splicing complexes (21,32). SR-25, as other SR proteins, is also rich in potential phosphorylation residues and motifs (29). Previous studies demonstrated that a specific conformation of the SR proteins is required for their protein-protein interactions or for their phosphorylation/dephosphorylation during the splicing cycle (32). However, the highly repetitive sequence composition and the presence of multiple proline residues in the SR domains of SR proteins indicate that they are rather unstructured (33). Therefore, SR proteins require certain chaperones to mediate their conformational changes in the spliceosome (33). As a multifunctional chaperone, CypA could act specifically to alter protein conformations (4). In addition, the present study demonstrated that the disruption of the PPIase domain affected the interaction of CypA with SR-25, indicating that this interaction may depend on the PPIase domain.

The present study revealed that CypA could induce the expression of SR-25 when CypA was overexpressed in Hep3B cells. Furthermore, the mRNA levels of CypA and SR-25 in HCC indicated that the expression of CypA exhibited a significant correlation with that of SR-25 in HCC tissue. A previous study indicated that SR-25 is one of the mediators in the Ras-related C3 botulinum toxin substrate (Rac)1 signaling pathway (34). Upregulated SR-25 in dominant negative mutant of Rac1 (Rac1N17) cells reduces the apoptosis sensitivity toward paclitaxel of melanoma cells, suggesting a role in the regulation of apoptosis (34). As CypA expression was also observed to be upregulated in paclitaxel-resistant cancer cells (35), it can be speculated that the interaction of CypA with SR-25 may participate in the regulation of apoptosis in HCC. However, whether there is a signaling loop that involves CypA, SR-25 and Rac1 in the regulation of apoptosis requires further investigation.

In conclusion, for the first time, the present study revealed a novel CypA-binding protein, SR-25. The present study revealed that CypA could induce the expression of SR-25 in Hep3B cells, and that this interaction may depend on the PPIase domain of CypA. These results suggested that the interaction between CypA and SR-25 proteins may participate in potential carcinogenic functions of CypA in HCC. Additionally, there was a significant correlation between the expression of CypA and that of SR-25 in HCC. Whether there is a signaling loop that involves CypA, SR-25 and Rac1 in the regulation of apoptosis in HCC requires further study. In addition, the potential therapeutic value of these two proteins for HCC is worth further investigation.

Acknowledgements

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