

• LIVER CANCER •

Expressions of cysteine-rich61, connective tissue growth factor and Nov genes in hepatocellular carcinoma and their clinical significance

Zhi-Jun Zeng, Lian-Yue Yang, Xiang Ding, Wei Wang

Zhi-Jun Zeng, Lian-Yue Yang, Xiang Ding, Wei Wang, Liver Cancer Laboratory, Department of Surgery, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Supported by the National Key Technologies R and D Program, No. 2001BA703BO4 and the National Natural Science Foundation of China, No.30371595

Correspondence to: Lian-Yue Yang, Liver Cancer Laboratory, Department of Surgery, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China. lianyueyang@hotmail.com

Telephone: +86-731-4327326 **Fax:** +86-731-4327332

Received: 2004-02-28 **Accepted:** 2004-03-04

Abstract

AIM: To investigate the expression of cysteine-rich61 (Cyr61), connective tissue growth factor (CTGF) and nephroblastoma overexpressed gene (Nov) in hepatocellular carcinoma (HCC), and to evaluate the relationship between Cyr61, CTGF and Nov genes expression with invasion and metastasis of HCC.

METHODS: Thirty-one HCC specimens were divided into small hepatocellular carcinoma (SHCC), nodular hepatocellular carcinoma (NHCC), solitary large hepatocellular carcinoma (SLHCC) according to their diameter and number of nodes. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect the mRNA expression levels of Cyr61, CTGF and Nov genes in 31 resected specimens of hepatocellular carcinoma and para-cancerous normal liver tissues semi-quantitatively and the relation between their expression levels and clinical pathological parameters were compared.

RESULTS: The expressions of Cyr61 and CTGF mRNA in carcinoma tissues were significantly higher than those in para-cancerous normal liver tissues ($P < 0.01$). The expressions of Cyr61 and CTGF mRNA in HCC with venous invasion were higher than those in HCC without venous invasion. CTGF expression in HCC Edmondson's grade III-IV was significantly higher than that in HCC Edmondson's grade I-II ($P = 0.022$). There was no obvious correlation between Nov mRNA and clinical-pathological features. Compared to NHCC, SLHCC had better cell differentiation, easier capsule formation, less microscopic venous invasion, milder liver cirrhosis. The expressions of Cyr61 and CTGF mRNA in NHCC were significantly higher than those in SLHCC and SHCC.

CONCLUSION: Cyr61 and CTGF genes may play an important role in hepatocellular carcinogenesis and correlate with recurrence and metastasis of hepatocellular carcinoma. SLHCC has better biological behaviors than NHCC.

Zeng ZJ, Yang LY, Ding X, Wang W. Expressions of cysteine-rich61, connective tissue growth factor and Nov genes in hepatocellular carcinoma and their clinical significance. *World J Gastroenterol* 2004; 10(23): 3414-3418
<http://www.wjgnet.com/1007-9327/10/3414.asp>

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant cancers in the world, and the fourth most common cause of death from cancer, the second-leading cause of cancer death in China, which alone accounts for 53% of all liver cancer deaths worldwide. Although HCC resection plays an important role in improving HCC prognosis, it has been generally accepted that the high incidence of recurrence and metastasis is the most crucial prognostic factor in patients with HCC^[1]. The high recurrent rate in the liver with mainly intrahepatic metastatic spread remains a major obstacle to further improvement in the long-term survival after curative HCC resection^[2]. The mechanism of recurrence and metastasis of hepatocellular carcinoma is very complicated, and includes cell adhesion, matrix degradation, cell migration and angiogenesis^[3-7].

CCN gene family is a group of growth factor-inducible immediate-early genes, including cysteine-rich61 (Cyr61), connective tissue growth factor (CTGF), nephroblastoma overexpressed gene (Nov), Wnt-1 induced secreted protein 1 (WISP-1), WISP-2, WISP-3^[8,9]. CCN proteins are secreted extracellular matrix (ECM)-associated proteins that regulate cellular processes, such as adhesion, migration, mitogenesis, differentiation and survival^[10]. They also regulate more complex biological processes such as angiogenesis, chondrogenesis, tumorigenesis, fibrotic and vascular diseases^[11-13]. Cyr61 gene was originally identified as an immediately-early gene of mouse 3T3 fibroblasts, and was also found to be expressed in developing mouse cartilaginous elements and placental tissues^[14]. CTGF was originally identified in the conditioned culture medium of human umbilical vein endothelial cells, and revealed to be induced by transforming growth factor in human skin fibroblasts. Nov gene was identified as an aberrantly expressed gene in avian nephroblastomas induced by myeloblastosis-associated viruses. The overexpression of Nov gene was reported relative to human Wilms' tumors.

Taking cues from clinical observations and results of our laboratory researches, we have hypothesized that solitary large hepatocellular carcinoma (SLHCC) possesses relatively better biological behaviors^[15]. Furthermore, we have preliminarily proved our hypothesis by a series of researches^[16]. The clinical pathological features of SLHCC were better than nodular hepatocellular carcinoma (NHCC) and the molecular biological study also suggested that SLHCC possessed better molecular pathological features.

Cyr61, CTGF and Nov gene may overexpress in HCCs. In this report, we studied the expressions of Cyr61, CTGF and Nov genes in HCCs and para-cancerous normal liver tissues, to clarify whether these genes might play an important role in the recurrence and metastasis of HCCs. Furthermore, we examined the expressions of Cyr61, CTGF and Nov genes in SLHCC, NHCC and SHCC and compared their differences.

MATERIALS AND METHODS

Patients and tissue preparation

Thirty-one fresh HCC specimens and corresponding para-cancerous liver tissues were obtained by surgical resection at

Xiangya Hospital between March 2002 and March 2003. The patients with HCC consisted of 26 men and 5 women and the age of them ranged from 21 to 69 years (mean, 48 years). The patients were classified as SHCC (tumor largest diameter ≤ 5 cm for a single tumor nodule or the sum of diameters ≤ 5 cm for two tumor nodules), SLHCC (a single tumor nodule and tumor largest diameter >5 cm), NHCC (the nodules of tumor ≥ 2 , only two tumor nodules and the sum of diameters ≤ 5 cm were excluded). Furthermore, we divided 31 specimens into six groups: tumors <5 cm diameter and ≥ 5 cm, grade I-II and grade III-IV, liver cirrhosis and no liver cirrhosis, capsule formation and no capsule formation, microscopic portal vein tumor thrombosis and no microscopic portal vein tumor thrombosis. All specimens were examined under a microscope after haematoxylin and eosin (HE) staining.

RNA extraction and RT-PCR

Total RNA was isolated using Trizol reagent (GIBCO BRL, USA) and cDNA was synthesized from RNA by M-MLV reverse transcriptase (Promega, USA) with oligo-dT primers (Sango Technology, China). The primer sequences of Cyr61, CTGF and Nov genes were as follows: Cyr61, upstream: 5'-ACTTCATGGTCCCAGTGC GC-3', downstream: 5'-AAATCCGGGTTTCTTTCACA-3'; CTGF, upstream: 5'-GCAGGCTAGAGAAGCAGAGC-3', downstream: 5'-ATGCTCTTCATGCTGGTGCAG-3'; Nov, upstream: 5'-AGCATGCAGAGTGTGCAGAG-3', downstream: 5'-GGTGTGCCACTTACCTGTCC-3'; β -actin, upstream: 5'-CTGCAATCCGAAAGAAGCTG-3', downstream: 5'-ATCTTCAAACCTCCATGATG-3'. The conditions of PCR were as follows: after an initial denaturation at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 1 min and extension at 72 °C for 1 min. The bands representing amplified products were analyzed by Stratagene Eagle-eye scanner. Expressions of Cyr61, CTGF and Nov genes were presented by the relative yield of the PCR products of the target sequence to that of the β -actin gene.

Statistical analysis

The results of RT-PCR were statistically analyzed using the Student's *t* test. Fisher's exact test was used to determine the relationship between the expressions of Cyr61, CTGF and Nov genes and clinicopathological characteristics of HCCs. SPSS11.0 software was used. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of Cyr61, CTGF and Nov mRNA in HCC and para-cancerous liver tissues

The expressions of Cyr61 and CTGF mRNA in HCC tissues were significantly higher than those in para-cancerous normal liver tissues. The expression of Nov gene was higher than that in para-cancerous normal liver tissues (Table 1). The difference in Nov gene expression between these two groups did not reach statistical significance. The expressions of Cyr61, CTGF and Nov genes are shown in Figure 1.

Table 1 Expression of Cyr61, CTGF and Nov mRNA in HCC and para-cancerous liver tissues (mean \pm SD)

	<i>n</i>	Cyr61	CTGF	Nov
HCC	31	2.34 \pm 0.46 ^b	2.21 \pm 0.34 ^b	1.56 \pm 0.21
Para-cancerous	31	0.48 \pm 0.29	0.65 \pm 0.33	0.89 \pm 0.64

^b $P < 0.01$ vs para-cancerous tissues.

Between the groups with different pathological characteristics, the expressions of Cyr61, CTGF and Nov genes were significantly different. Especially, the differences between the two groups with and without microscopic portal vein tumor thrombosis were of great significance. Moreover, CTGF expression was significantly different between Edmondson's grades I-II and III-IV. The relationship between the expression of Cyr61, CTGF, Nov mRNA and clinicopathological features of HCC patients is shown in Table 2.

Clinical and pathological features of three types of HCC

Thirty-one HCC specimens were divided into SLHCC, SHCC and NHCC according to their diameter and number of nodules. Liver cirrhosis, microvascular invasion, capsule formation, Edmondson's classification of HCC were studied in each group. NHCC group had a higher incidence of microvascular invasion compared with SLHCC and SHCC ($P < 0.05$). Only 8.3% of NHCCs were classified as Edmondson's grade I-II, while 62.5% of SLHCC and 63.6% of SHCC were classified as Edmondson's grade I-II. The differentiation of NHCC was significantly poorer than that of SLHCC and SHCC ($P < 0.05$). The other three pathological features of SLHCC and SHCC were also better than NHCC but did not reach statistical significance. No statistical difference in the five pathological features was observed between SLHCC and SHCC (Table 3).

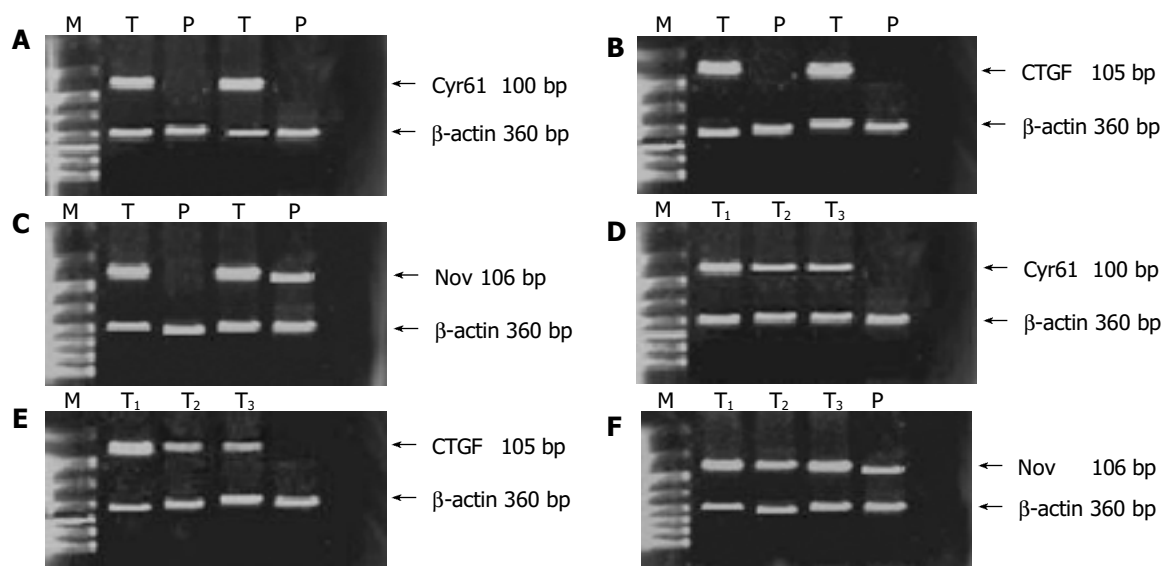


Figure 1 Expressions of Cyr61, CTGF and Nov mRNA in hepatocellular carcinoma A and D: Cyr61 mRNA expression, product size 100 bp; B and E: CTGF mRNA expression, product size 105 bp; C and F: Nov mRNA expression, product size 106 bp; M, DNA marker; T, tumor; P, para-cancerous normal liver tissues; T₁, NHCC; T₂, SLHCC; T₃, SHCC. PCR product of β -actin was 360 bp.

Table 2 Relationship between Cyr61, CTGF and Nov mRNA expressions and clinicopathological features (mean±SD)

	<i>n</i>	Cyr61	<i>P</i>	CTGF	<i>P</i>	Nov	<i>P</i>
PV thrombosis							
Present	16	2.59±0.41	0.024	2.41±0.39	0.031	1.45±0.56	0.147
Absent	15	2.08±0.65		2.10±0.58		1.56±0.45	
AFP level							
≤20 ng/mL	19	2.26±0.48	0.068	2.21±0.20	0.063	1.38±0.43	0.241
>20 ng/mL	12	2.36±0.71		2.23±0.84		1.66±0.71	
Tumor size (cm)							
≤ 5 cm	20	2.35±0.86	0.124	2.26±0.79	0.071	1.59±0.58	0.251
> 5 cm	11	2.32±0.41		2.09±0.53		1.55±0.31	
Capsule formation							
Positive	18	2.35±0.34	0.136	2.31±0.34	0.132	1.87±0.65	0.135
Negative	13	2.33±0.67		2.15±0.87		1.36±0.81	
Histological grade							
I-II	13	2.21±0.39	0.052	1.89±0.54	0.022	1.35±0.47	0.132
III-IV	18	2.51±0.41		2.51±0.61		1.58±0.68	

PV: portal vein.

Table 3 Pathological features of three types of HCC

Pathological features	<i>n</i>	SHCC (<i>n</i> =11)	NHCC (<i>n</i> =12)	SLHCC (<i>n</i> =8)
Microvascular invasion				
Present	16	3	10	3 ^a
Absent	15	8	2	5
Capsule formation				
Present	18	8	4	6
Absent	13	3	8	2
Edmondson's classification				
I-II	13	7	1	5 ^a
III-IV	18	4	11	3
Liver cirrhosis				
Present	9	4	9	2
Absent	22	7	3	6
AFP concentration				
≤20 ng/mL	19	6	9	4
>20 ng/mL	12	5	3	4

^a*P*<0.05 vs NHCC.**Expression of Cyr61, CTGF and Nov mRNA in three types of HCCs**

The expression of Cyr61 mRNA in NHCC was significantly higher than that in SLHCC and SHCC (*P* = 0.024 and *P* = 0.031, respectively). The expression of CTGF mRNA in nodular HCC was also significantly higher than that in SLHCC and SHCC (*P* = 0.016 and *P* = 0.027, respectively). No statistical difference in the expression of Nov gene among NHCC, SLHCC and SHCC was observed (Table 4).

DISCUSSION

All members of the CCN gene family possess a secretory signal peptide at the N terminus, indicating that they are secreted proteins. Several lines of evidence supported a role of CCN molecules in tumorigenesis^[8].

Cyr61 is a secreted, 40-kDa, cysteine-rich and heparin-binding protein coded by a growth factor-inducible immediate early gene^[14]. Recently, it has been reported as an angiogenic inducer that can promote tumor growth and vascularization^[17]. A mechanistic framework for the biological properties of Cyr61 has been provided by the finding that Cyr61 binds to integrin α_vβ₃, which represents the first molecularly defined receptor for any member of the CCN family^[18]. Interaction of α_vβ₃ with Cyr61 may account for its promotion of chemotaxis and growth factor-mediated DNA synthesis as well as cell adhesion since integrins have been known to modulate cell migration and growth factor signaling in other systems^[16,17]. In a direct interaction between the two molecules, Cyr61-mediated adhesion and migration of cultured endothelial cells were specifically inhibited by the peptide RGDS and/or antiintegrin α_vβ₃^[19-23]. In addition to its integrin-binding property, Cyr61 appears to be localized to its site of synthesis by associating with the ECM, possibly by binding to heparin-like molecules. This interaction could limit the extent of Cyr61 diffusion so that its site of action is in close proximity of its site of synthesis^[24]. In our study, the expression of Cyr61 gene in HCC tissue was markedly higher than that in para-cancerous normal liver tissues, indicating that Cyr61 may play an important role in hepatocellular carcinogenesis.

CTGF is a cysteine-rich mitogenic peptide that was originally identified as a growth factor secreted by vascular endothelial cells^[25]. It was selectively induced in fibroblasts after activation with TGF^[26]. A previous study demonstrated the coordinate expression of TGF1 and CTGF in granulation beds during wound

Table 4 Cyr61, CTGF and Nov mRNA expressions in three types of HCCs

HCC	<i>n</i>	Cyr61	<i>P</i>	CTGF	<i>P</i>	Nov	<i>P</i>
SLHCC	8	2.22±0.36		2.09±0.44		1.52±0.41	
NHCC	12	2.51±0.53	0.037 ^a	2.38±0.29	0.043 ^a	1.58±0.36	0.287
SHCC	11	2.18±0.42		2.03±0.31		1.46±0.19	

^a*P*<0.05 vs SLHCC.

repair, and found that dermal fibroblasts in scleroderma lesions overexpressed CTGF^[27]. In addition to contributing to TGF- β -mediated AIG, CTGF could interact synergistically with EGF, PDGF, IGF-I, or bFGF, suggesting that it activates the receptors and/or signaling pathways used by other growth factors^[28,29]. Consistent with its profibrotic properties, CTGF has been found to be overexpressed in pancreatic cancers, mammary tumors, and melanomas^[30-32]. In our study, the expression of CTGF gene in HCC tissue was obviously higher than that in para-cancerous normal liver tissues, indicating that CTGF might play an important role in hepatocellular carcinogenesis.

Nov gene was first recognized as an overexpressed gene in nephroblastomas induced by myeloblastosis-associated virus type 1^[33]. Unlike the other members of this family, Nov gene expression was associated with quiescence and transcriptionally downregulated upon expression of p60 v-src in RSV-infected CEF^[34]. It has been reported that overexpression of normal Nov gene in CEF has an inhibitory effect on cell growth, whereas expression of an amino-terminal truncated form of Nov gene was able to induce morphological transformation^[35,36]. Therefore, Nov is a negative regulator of cell growth, the amino-truncation of which would result in oncogenic activation^[37,38]. Also, Koliopoulos *et al.*^[42] reported that Nov was a ligand of integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, and acted directly upon endothelial cells to stimulate pro-angiogenic activities, thus inducing angiogenesis *in vivo*. While, in our study, the expression of Nov gene in HCC and para-cancerous normal liver tissues had no difference. The diagnostic significance of Nov gene expression in HCC needs to be further investigated with more samples.

Metastasis and invasion of HCC is a multistep process, the molecular and cellular mechanisms of which have not been fully understood^[39]. They may involve matrix degradation, cell motility, angiogenesis, *etc.* CCN proteins could regulate biological processes such as angiogenesis, chondrogenesis, tumorigenesis, fibrotic and vascular diseases^[4-6,40]. Numerous *in vitro* studies indicated that Cyr61 protein was related to angiogenesis^[41]. Overexpressions of Cyr61, CTGF and Nov genes have been found in metastatic lesions of esophageal cancer, breast cancer, colon tumors, lung cancer and osteosarcoma^[42-45]. In the present study, we statistically analyzed the clinical and pathological parameters. No significant correlation was found among the parameters of age, sex, cause of liver diseases. Cyr61 and CTGF mRNA levels in patients with portal vein tumor thrombosis were significantly higher compared to those in patients without portal vein invasion. Moreover, CTGF mRNA level in Edmondson's grade III-IV was significantly higher than that in Edmondson's grade I-II. These results indicated that Cyr61 and CTGF had a close relationship with invasion and metastasis of HCC.

Previous studies have shown that SLHCC is different from other types of HCC in pathological features and invasiveness^[16]. Coordinated with this, the relatively better pathological features of SHCC were found in this study. The differentiation of SLHCC was much better than that of NHCC, and microvascular invasion was observed more frequently in NHCC compared with SLHCC. No statistical difference in pathological features was observed between SHCC and SLHCC. In addition, the transcription level of Cyr61 and CTGF in NHCC was much higher than that in SLHCC and SHCC, while no statistical difference was observed between SLHCC and SHCC. The lower transcription of Cyr61 and CTGF mRNA in SLHCC was probably due to the relatively better molecular pathological features of SLHCC.

Our findings indicate that Cyr61 and CTGF genes are related to tumorigenesis of HCC, and may enhance the invasion and metastasis of HCC. Its molecular basis remains to be elucidated. What are the most important factors regulating the expression level of CCN family and how does CCN gene family regulate effector protein will be the subjects of our future studies. When

the upstream and downstream signaling pathways are understood, those findings will provide new potential tools for the prognosis or prevention of invasion and metastasis of HCC.

REFERENCES

- 1 Zhou XD. Recurrence and metastasis of hepatocellular carcinoma: progress and prospects. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 35-41
- 2 Nakashima Y, Nakashima O, Tanaka M, Okuda K, Nakashima M, Kojiro M. Portal vein invasion and intrahepatic micrometastasis in small hepatocellular carcinoma by gross type. *Hepatol Res* 2003; **26**: 142-147
- 3 Shiratori Y, Yoshida H, Omata M. Management of hepatocellular carcinoma: advances in diagnosis, treatment and prevention. *Expert Rev Anticancer Ther* 2001; **1**: 277-290
- 4 Ariizumi S, Takasaki K, Yamamoto M, Ohtsubo T, Katsuragawa H, Katagiri S. Histopathologic differentiation of the main nodule determines outcome after hepatic resection for synchronous multicentric hepatocellular carcinomas. *Hepatogastroenterology* 2004; **51**: 500-504
- 5 McGlynn KA, Edmonson MN, Michielli RA, London WT, Lin WY, Chen GC, Shen FM, Buetow KH. A phylogenetic analysis identifies heterogeneity among hepatocellular carcinomas. *Hepatology* 2002; **36**: 1341-1348
- 6 Murawaki Y, Ikuta Y, Okamoto K, Mimura K, Koda M, Kawasaki H. Plasma matrix metalloproteinase-9 (gelatinase B) in patients with hepatocellular carcinoma. *Res Commun Mol Pathol Pharmacol* 2000; **108**: 351-357
- 7 Mukai M, Nakamura H, Tatsuta M, Iwasaki T, Togawa A, Imamura F, Akedo H. Hepatoma cell migration through a mesothelial cell monolayer is inhibited by cyclic AMP-elevating agents via a Rho-dependent pathway. *FEBS Lett* 2000; **484**: 69-73
- 8 Bork P. The modular architecture of a new family of growth regulators related to connective tissue growth factor. *FEBS Lett* 1993; **327**: 125-130
- 9 Brigstock DR, Goldschmeding R, Katsube KI, Lam SC, Lau LF, Lyons K, Naus C, Perbal B, Riser B, Takigawa M, Yeger H. Proposal for a unified CCN nomenclature. *Mol Pathol* 2003; **56**: 127-128
- 10 Kireeva ML, MO FE, Yang GP, Lau LF. Cyr61, a product of a growth factor-inducible immediate-early gene, promotes cell proliferation, migration, and adhesion. *Mol Cell Biol* 1996; **16**: 1326-1334
- 11 Hadjiargyrou M, Ahrens W, Rubin CT. Temporal expression of the chondrogenic and angiogenic growth factor CYR61 during fracture repair. *J Bone Miner Res* 2000; **15**: 1014-1023
- 12 Brigstock DR, Steffen CL, Kim GY, Vegunta RK, Diehl JR, Harding PA. Purification and characterization of novel heparin-binding growth factors in uterine secretory fluids. Identification as heparin-regulated M_r 10000 forms of connective tissue growth factor. *J Biol Chem* 1997; **272**: 20275-20282
- 13 Lake AC, Bialik A, Walsh K, Castellot JJ Jr. CCN5 is a growth arrest-specific gene that regulates smooth muscle cell proliferation and motility. *Am J Pathol* 2003; **162**: 219-231
- 14 O'Brien TP, Lau LF. Expression of the growth factor-inducible immediate early gene *cyr61* correlates with chondrogenesis during mouse embryonic development. *Cell Growth Differ* 1992; **3**: 645-654
- 15 Yang LY, Huang GW. Surgical strategy of large hepatocellular carcinoma. *Linchuang Waikie Zazhi* 2001; **9**: 4-5
- 16 Liu HL, Yang LY, Huang GW, Yang JQ. The effect of integrin α_v subunit on the angiogenesis, invasiveness and metastasis of hepatocellular carcinoma. *Zhonghua Putong Waikie Zazhi* 2002; **9**: 542-543
- 17 Inoki I, Shiomi T, Hashimoto G, Enomoto H, Nakamura H, Makino K, Ikeda E, Takata S, Kobayashi K, Okada Y. Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. *FASEB J* 2002; **16**: 219-221
- 18 Leng E, Malcolm T, Tai G, Estable M, Sadowski I. Organization and expression of the Cyr61 gene in normal human fibroblasts. *J Biomed Sci* 2002; **9**: 59-67
- 19 Grzeszkiewicz TM, Kirschling DJ, Chen N, Lau LF. CYR61

- stimulates human skin fibroblast migration through Integrin alpha vbeta 5 and enhances mitogenesis through integrin alpha vbeta 3, independent of its carboxyl-terminal domain. *J Biol Chem* 2001; **276**: 21943-21950
- 20 **Fataccioli V**, Abergel V, Wingertsmann L, Neuville P, Spitz E, Adnot S, Calenda V, Teiger E. Stimulation of angiogenesis by Cyr61 gene: a new therapeutic candidate. *Hum Gene Ther* 2002; **13**: 1461-1470
- 21 **Hilfiker A**, Hilfiker-Kleiner D, Fuchs M, Kaminski K, Lichtenberg A, Rothkotter HJ, Schieffer B, Drexler H. Expression of CYR61, an angiogenic immediate early gene, in arteriosclerosis and its regulation by angiotensin II. *Circulation* 2002; **106**: 254-260
- 22 **Kothapalli D**, Grotendorst GR. CTGF modulates cell cycle progression in cAMP-arrested NRK fibroblasts. *J Cell Physiol* 2000; **182**: 119-126
- 23 **Schober JM**, Chen N, Grzeszkiewicz TM, Jovanovic I, Emeson EE, Ugarova TP, Ye RD, Lau LF, Lam SC. Identification of integrin alpha (M) beta (2) as an adhesion receptor on peripheral blood monocytes for Cyr61 (CCN1) and connective tissue growth factor (CCN2): immediate-early gene products expressed in atherosclerotic lesions. *Blood* 2002; **99**: 4457-4465
- 24 **Tamura I**, Rosenbloom J, Macarak E, Chaqour B. Regulation of Cyr61 gene expression by mechanical stretch through multiple signaling pathways. *Am J Physiol Cell Physiol* 2001; **281**: C1524-1532
- 25 **Surveyor GA**, Wilson AK, Brigstock DR. Localization of connective tissue growth factor during the period of embryo implantation in the mouse. *Biol Reprod* 1998; **59**: 1207-1213
- 26 **Wang JF**, Olson ME, Reno CR, Wright JB, Hart DA. The pig as a model for excisional skin wound healing: characterization of the molecular and cellular biology, and bacteriology of the healing process. *Comp Med* 2001; **51**: 341-348
- 27 **Maquart FX**, Chastang F, Simeon A, Birembaut P, Gillery P, Wegrowski Y. Triterpenes from *Centella asiatica* stimulate extracellular matrix accumulation in rat experimental wounds. *Eur J Dermatol* 1999; **9**: 289-296
- 28 **Denton CP**, Abraham DJ. Transforming growth factor-beta and connective tissue growth factor: key cytokines in scleroderma pathogenesis. *Curr Opin Rheumatol* 2001; **13**: 505-511
- 29 **Ehrchen J**, Heuer H, Sigmund R, Schafer MK, Bauer K. Expression and regulation of osteopontin and connective tissue growth factor transcripts in rat anterior pituitary. *J Endocrinol* 2001; **169**: 87-96
- 30 **Wenger C**, Ellenrieder V, Alber B, Lacher U, Menke A, Hameister H, Wilda M, Iwamura T, Beger HG, Adler G, Gress TM. Expression and differential regulation of connective tissue growth factor in pancreatic cancer cells. *Oncogene* 1999; **18**: 1073-1080
- 31 **Frazier KS**, Grotendorst GR. Expression of connective tissue growth factor mRNA in the fibrous stroma of mammary tumors. *Int J Biochem Cell Biol* 1997; **29**: 153-161
- 32 **Kubo M**, Kikuchi K, Nashiro K, Kakinuma T, Hayashi N, Nanko H, Tamaki K. Expression of fibrogenic cytokines in desmoplastic malignant melanoma. *Br J Dermatol* 1998; **139**: 192-197
- 33 **Perbal B**. NOV (nephroblastoma overexpressed) and the CCN family of genes: structural and functional issues. *Mol Pathol* 2001; **54**: 57-79
- 34 **Babic AM**, Kireeva ML, Kolesnikova TV, Lau LF. CYR61, a product of a growth factor-inducible immediate early gene, promotes angiogenesis and tumor growth. *Proc Natl Acad Sci U S A* 1998; **95**: 6355-6360
- 35 **Xie D**, Nakachi K, Wang H, Elashoff R, Koeffler HP. Elevated levels of connective tissue growth factor, WISP-1, and CYR61 in primary breast cancers associated with more advanced features. *Cancer Res* 2001; **61**: 8917-8923
- 36 **Sakamoto K**, Yamaguchi S, Ando R, Miyawaki A, Kabasawa Y, Takagi M, Li CL, Perbal B, Katsube K. The nephroblastoma overexpressed gene (NOV/ccn3) protein associates with Notch1 extracellular domain and inhibits myoblast differentiation via Notch signaling pathway. *J Biol Chem* 2002; **277**: 29399-29405
- 37 **Kocialkowski S**, Yeger H, Kingdom J, Perbal B, Schofield PN. Expression of the human NOV gene in first trimester fetal tissues. *Anat Embryol* 2001; **203**: 417-427
- 38 **Lin CG**, Leu SJ, Chen N, Tebeau CM, Lin SX, Yeung CY, Lau LF. CCN3 (NOV) is a novel angiogenic regulator of the CCN protein family. *J Biol Chem* 2003; **278**: 24200-24208
- 39 **Zhao ZC**, Zheng SS, Wan YL, Jia CK, Xie HY. The molecular mechanism underlying angiogenesis in hepatocellular carcinoma: the imbalance activation of signaling pathways. *Hepatobiliary Pancreat Dis Int* 2003; **2**: 529-536
- 40 **Chaqour B**, Whitbeck C, Han JS, Macarak E, Horan P, Chichester P, Levin R. Cyr61 and CTGF are molecular markers of bladder wall remodeling after outlet obstruction. *Am J Physiol Endocrinol Metab* 2002; **283**: E765-774
- 41 **Wenger C**, Ellenrieder V, Alber B, Lacher U, Menke A, Hameister H, Wilda M, Iwamura T, Beger HG, Adler G, Gress TM. Expression and differential regulation of connective tissue growth factor in pancreatic cancer cells. *Oncogene* 1999; **18**: 1073-1080
- 42 **Koliopoulos A**, Friess H, di Mola FF, Tang WH, Kubulus D, Brigstock D, Zimmermann A, Buchler MW. Connective tissue growth factor gene expression alters tumor progression in esophageal cancer. *World J Surg* 2002; **26**: 420-427
- 43 **Pennica D**, Swanson TA, Welsh JW, Roy MA, Lawrence DA, Lee J, Brush J, Taneyhill LA, Deuel B, Lew M, Watanabe C, Cohen RL, Melhem MF, Finley GG, Quirke P, Goddard AD, Hillan KJ, Gurney AL, Botstein D, Levine AJ. WISP genes are members of the connective tissue growth factor family that are up-regulated in wnt-1- transformed cells and aberrantly expressed in human colon tumors. *Proc Natl Acad Sci U S A* 1998; **95**: 14717-14722
- 44 **Astolfi A**, De Giovanni C, Landuzzi L, Nicoletti G, Ricci C, Croci S, Scopece L, Nanni P, Lollini PL. Identification of new genes related to the myogenic differentiation arrest of human rhabdomyosarcoma cells. *Gene* 2001; **274**: 139-149
- 45 **Sampath D**, Winneker RC, Zhang Z. Cyr61, a member of the CCN family, is required for MCF-7 cell proliferation: regulation by 17beta-estradiol and overexpression in human breast cancer. *Endocrinology* 2001; **142**: 2540-2548