Chinese Journal of Physiology 54(3): 161-168, 2011
DOI: 10.4077/CJP.2011.AMM021

Expression of LGR8 and Related Biomarkers in Hepatocellular Carcinoma: Correlation with Clinicopathological Parameters

Chih-Kung Lin, Jong-Shiaw Jin, Cheng-Ping Yu, and Wen-Chiuan Tsai

Department of Pathology, Tri-Service General Hospital, National Defense Medical Center Taipei, Taiwan, Republic of China

Abstract

This study aimed to evaluate the relationship between expression of LGR8, VEGF, MMP-2, MMP-9, fascin-1 and cortactin with clinicopathological parameters in hepatocellular carcinoma (HCC). The six biomarkers were investigated immunohistochemically using tissue microarrays of 93 HCC specimens. The tumor cells showed significant expression of LGR8, VEGF, MMP-9, fascin-1 and cortactin, but not of MMP-2. In addition, higher immunostaining scores for LGR8 in HCC showed negative correlation with T and AJCC clinical stages and upregulation of MMP-9, but no correlation with poorer survival rate; cortactin expression is correlated with poorer tumor differentiation in HCC. Thus, our data suggest that higher expression of LGR8 may facilitate tumor invasiveness in the early clinical stage of hepatocellular carcinoma, and synergic effects of cortactin also play a crucial role in the intrahepatic metastasis. Although tumor biological evidence implicates the relaxin-like hormone family as endocrine mediators of critical cellular action in cancer, characterization of target molecules and signaling pathways specific for LGR8 in defined tumor entities and crosstalk of the relaxin receptors with other receptor systems relevant to carcinogenesis will be of significant clinical relevance and may contribute to novel therapeutic strategies against hepatocellular carcinoma.

Key Words: LGR8, VEGF, MMP-2, MMP-9, fascin-1, cortactin, immunostaining score

Introduction

Hepatocellular carcinoma (HCC) is the most common histological type of primary liver cancer, accounting for 7.4% and 3.2% of all male and female malignancies, respectively (21). Poor prognosis and therapeutic failure are identified in HCC patients, especially in HCC cases with portal thrombosis and TP53 mutation (2, 25). The heterodimeric peptide relaxin, a member of the insulin-like superfamily and one of the first peptide hormones to be discovered (9), is the founding member of the family of relaxin-like hormones. Relaxin, similar to its sister hormones insulin and the insulin-like growth factors, is now perceived as a multifunctional hormone (14). Relaxin and INSL3 have been identified in tumor tissues of cancers of the breast (10), thyroid gland (11), gastrointestinal tract (28) and the male reproductive system (16). Relaxin has been implicated in defined steps associated with carcinogenesis including tumor cell proliferation, differentiation, invasion and neovascularization (27).

LGR8, the recent landmark discovery of relaxin/INSL3 receptor, is a member of the family of leucine-rich repeat-containing G protein-coupled receptors (12). In recent studies, LGR8 has shown auto/paracrine relaxin-like action in tumor tissues leading to the elucidation of cellular pathways involved in the proposed functions of relaxin in tumor biology.

There is increasing evidence for the involvement

Corresponding author: Wen-Chiuan Tsai, M.D., Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, No. 325, Sec. 2, Cheng-Gong Road, Taipei, Taiwan, R.O.C. Tel: +886-2-87923311 ext. 16731, Fax: +886-2-87927159, E-mail: lueshear@seed.net.tw
Received: March 23, 2010; Revised: June 3, 2010; Accepted: August 9, 2010.
 ISSN : 0304-4920. http://www.cps.org.tw

of relaxin in tumor invasion. Cellular pathways involve the matrix metalloproteinases (MMP) and tissue inhibitors of MMP (TIMP) are important in normal and abnormal physiological actions of cellular migration and invasion. Upregulation of MMPs by relaxin may provide a possible mechanism for the indirect role of relaxin in cellular invasiveness (Fig. 4). Matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) have been associated with metastatic potentials facilitating tumor cell migration across basement membrane (29).

Vascular endothelial growth factor (VEGF), an angiogenic stimulator, has been associated with tumor growth and metastasis. Relaxin has been shown to upregulate VEGF in stromal and glandular epithelial cells of the endometrium during wound healing and VEGF and bFGF in the human myelomonocytic leukemia cell line, THP-1, which expresses relaxin receptors (22, 32, 33).

Fascin and cortactin are actin-binding proteins that are involved in the rearrangement of the cytoskeleton and facilitation of cellular motility (8, 34). Previous studies have shown that fascin is markedly upregulated in skin (5), breast (6), lung (23), colon (15), brain (24), ovary (13), urinary bladder (30) and gastric malignancies (31). The gene responsible for cortactin expression has been mapped in the chromosomal region 11q13 and is frequently amplified in some human cancers such as breast, head/neck carcinomas, gastric adenocarcinoma (20, 26, 31).

A direct involvement of relaxin/INSL3 receptor LGR8 in carcinogenesis remains to be demonstrated. This is the first report that tissue microarrays were used to demonstrate the expression and relationship of LGR8, VEGF, MMP-2, MMP-9, fascin-1 and cortactin with clinicopathological parameters of hepatocellular carcinoma. It was hoped that such an approach would provide the structural basis to elucidate the action and regulation of relaxin receptor-derived signal cascades in HCC.

Materials and Methods

Hepatocellular carcinoma tissue microarray slides were constructed from 93 paraffin-embedded primary tumors and 25 non-tumor normal liver tissues. Two experienced pathologists confirmed the pathological diagnosis in each case before the tissue array was constructed.

Histological differentiation (or clinical stage) was determined according to the TNM (WHO criteria). One core tissue sample was taken from a representative area of each paraffin-embedded tumor tissue, and the tissue microarray slides were constructed. Each representative core sample in the tissue microarray slide was 2 mm in diameter. None of the patients had received chemotherapy before surgery.

Immunohistochemistry

Tissue microarray sections were dewaxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 10 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating (100°C) each section for 30 min in 0.01 mol/L sodium citrate buffer (pH 6.0). After 3 rinses in phosphate buffered saline (PBS) for 5 min, each section was incubated for 1 h at room temperature with one of the following antibodies all diluted in PBS: mouse monoclonal antibodies to human fascin-1 (1:100 dilution; NeoMarkers, Freemont, CA, USA), human MMP-2 (1:100; NeoMarkers), human MMP-9 (1:100; NeoMarkers), and mouse polyclonal antibodies to human cortactin (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA), a mouse monoclonal antihuman VEGF antibody (1:50; clone VG1, DAKO, Carpinteria, CA, USA), and a mouse polyclonal to LGR8 antibody (1:100; abcam, Cambridge, MA, USA). The slides were washed 3 times in PBS for 5 min, incubated with horseradish peroxidase-labeled rabbit anti-mouse immunoglobulin (DAKO; 1 h at room temperature), washed 3 times and incubated with a solution of diaminobenzidine (DAB) at room temperature to visualize peroxidase activities.

Two experienced pathologists evaluated the immunoreactivity and histological appearance of all tissue samples in the microarray. The intensity of cytoplasmic and membrane tumor cell staining was scored on a scale of 0 to 3: with 0 being no staining, 1 as weak intensity, 2 as moderate intensity and 3 as strongest intensity, and the percentage of tumor cells with cytoplasmic or membranous staining at each intensity was estimated. The percentage of cells (from 0 to 100) at each intensity was multiplied by the corresponding intensity (from 0 to 3) to obtain an immunostaining score ranging from 0 to 300.

Statistical Analysis

All results are expressed as means ± standard error of the mean (SEM). Immunostaining scores of fascin-1, cortactin, MMP-2, MMP-9, VEGF and LGR8 in the HCC cases were compared to the score of the normal liver tissue. Student *t*-test and Pearson Product Correlation test were used to analyze the relationship between the expression of these six biomarkers and the clinicopathological parameters of the hepatocellular carcinomas. Statistical significance was defined as a P value of less than 0.05. In addition, survival time was calculated from the date of surgery to the date of death. Survival times of two groups divided on the basis of median cortactin immunostaining scores were compared. Survival analysis was done using the Kaplan-Meier survival test.

Table 1. Immunostaining scores for six biomarkers in hepatocellular carcinoma and normal liver cells

Biomarkers		Intensity	% Staining	Total score
Hepatocellular carcinoma (n = 93)				
Fascin-1	expression	0.10 ± 0.04*	0.12 ± 0.05*	0.20 ± 0.11
Cortactin	expression	1.16 ± 0.08*	57.45 ± 4.00*	84.88 ± 8.30*
LGR8	expression	0.25 ± 0.05*	3.08 ± 1.30*	3.48 ± 1.35*
VEGF	expression	1.82 ± 0.06*	87.85 ± 2.48*	160.81 ± 7.68*
MMP-2	expression	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MMP-9	expression	1.09 ± 0.04*	87.10 ± 2.50*	97.31 ± 4.03*
Normal liver cells (n = 25)				
Fascin-1	expression	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cortactin	expression	0.30 ± 0.15	0.60 ± 0.31	0.60 ± 0.31
LGR8	expression	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
VEGF	expression	0.90 ± 0.10	5.00 ± 0.75	5.50 ± 0.90
MMP-2	expression	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MMP-9	expression	0.40 ± 0.04	2.80 ± 1.32	2.80 ± 1.31

Data are the means ± standard error of the mean (SEM) of immunostaining scores for six biomarkers in hepatocellular carcinoma and normal liver cells. *: $P < 0.05$ *vs*. normal liver cells.

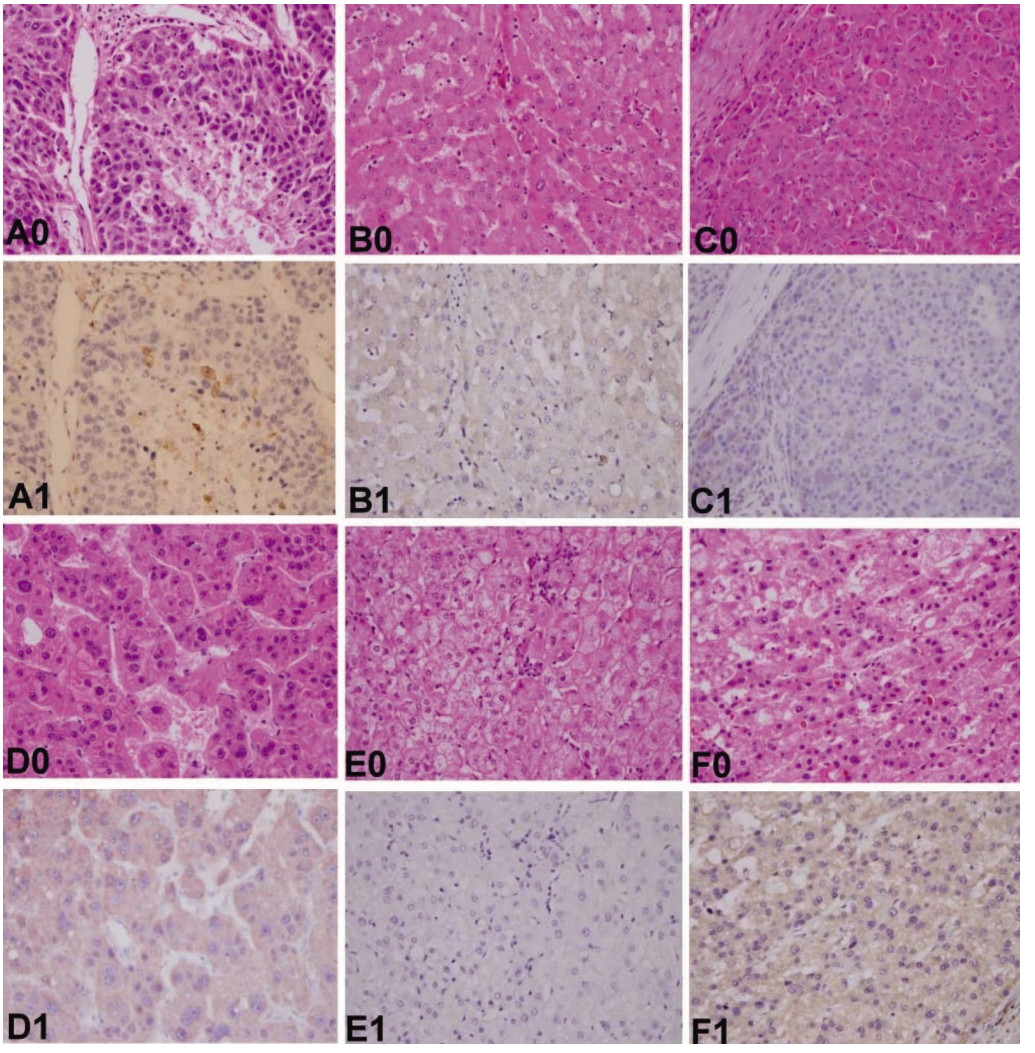

Fig. 1. Hematoxylin and Eosin (H&E) stain (A0, B0, C0, D0, E0, F0) and Fascin-1, cortactin, LGR8, VEGF, MMP-2 and MMP-9 staining of hepatocellular carcinomas (A1, B1, C1, D1, E1, F1). Original magnification, × 400.

Table 2. Total immunostaining scores of six biomarkers and clinicopathological parameters of hepatocellular carcinoma

		Fascin-1 total score			Cortactin total score			LGR8 total score		
	n	Mean	SEM[a]	Correlation	Mean	SEM	Correlation	Mean	SEM	Correlation
TNM stage										
T stage										
T1	58	0.10	0.08	r = 0.05	78.93	9.94	r = 0.10	3.78	1.65	r = -0.23*
T2	18	0.22	0.22		86.17	22.23		5.85	4.51	
T3	14	0.64	0.64		105.00	21.50		0.00	0.00	
T4	3	0.00	0.00		98.33	56.45		0.00	0.00	
N stage										
N0	93	0.20	0.11		84.88	8.30		3.48	1.35	
N1	0									
M stage										
M0	90	0.21	0.12	r = -0.04	83.38	8.48	r = 0.15	3.60	1.39	r = -0.09
M1	3	0.00	0.00		130.00	35.12		0.00	0.00	
AJCC stage										
I	58	0.10	0.08	r = 0.05	78.93	9.94	r = 0.08	3.78	1.65	r = -0.23*
II	17	0.25	0.25		78.19	23.90		6.56	5.06	
III	16	0.60	0.60		111.33	20.99		0.00	0.00	
IV	3	0.00	0.00		65.00	30.14		0.00	0.00	
Tumor differentiation										
Well	9	0.00	0.00	r = 0.13	58.00	12.87	r = 0.26*	3.33	2.36	r = -0.14
Moderate	62	0.10	0.07		75.44	9.92		3.37	1.55	
Poor	20	0.65	0.48		116.75	20.16		4.25	3.99	
Anaplastic	2	0.00	0.00		180.00	20.00		0.00	0.00	

		VEGF total score			MMP-2 total score			MMP-9 total score		
	n	Mean	SEM	Correlation	Mean	SEM	Correlation	Mean	SEM	Correlation
TNM stage										
T stage										
T1	58	167.41	9.71	r = -0.05	0.00	0.00		98.62	5.34	r = 0.03
T2	18	124.40	15.66		0.00	0.00		86.67	7.71	
T3	14	171.79	21.63		0.00	0.00		97.86	8.72	
T4	3	200.00	0.00		0.00	0.00		133.33	33.33	
N stage										
N0	93	160.81	7.68		0.00	0.00		97.31	4.03	
N1	0									
M stage										
M0	90	160.61	7.88	r = 0.02	0.00	0.00		97.22	4.17	r = 0.08
M1	3	166.67	33.31		0.00	0.00		100.00	0.00	
AJCC stage										
I	58	167.42	9.71	r = -0.04	0.00	0.00		98.62	5.34	r = 0.03
II	17	121.25	16.90		0.00	0.00		85.00	8.61	
III	16	173.67	20.23		0.00	0.00		98.00	8.12	
IV	3	200.00	0.00		0.00	0.00		133.33	33.30	
Tumor differentiation										
Well	9	155.56	27.03	r = 0.01	0.00	0.00		98.89	15.32	r = -0.08
Moderate	62	162.02	9.61		0.00	0.00		101.45	5.07	
Poor	20	166.00	15.63		0.00	0.00		83.50	7.23	
Anaplastic	2	95.00	5.00		0.00	0.00		100.00	0.00	

a: SEM: Standard Error of Mean

*: $P < 0.05$

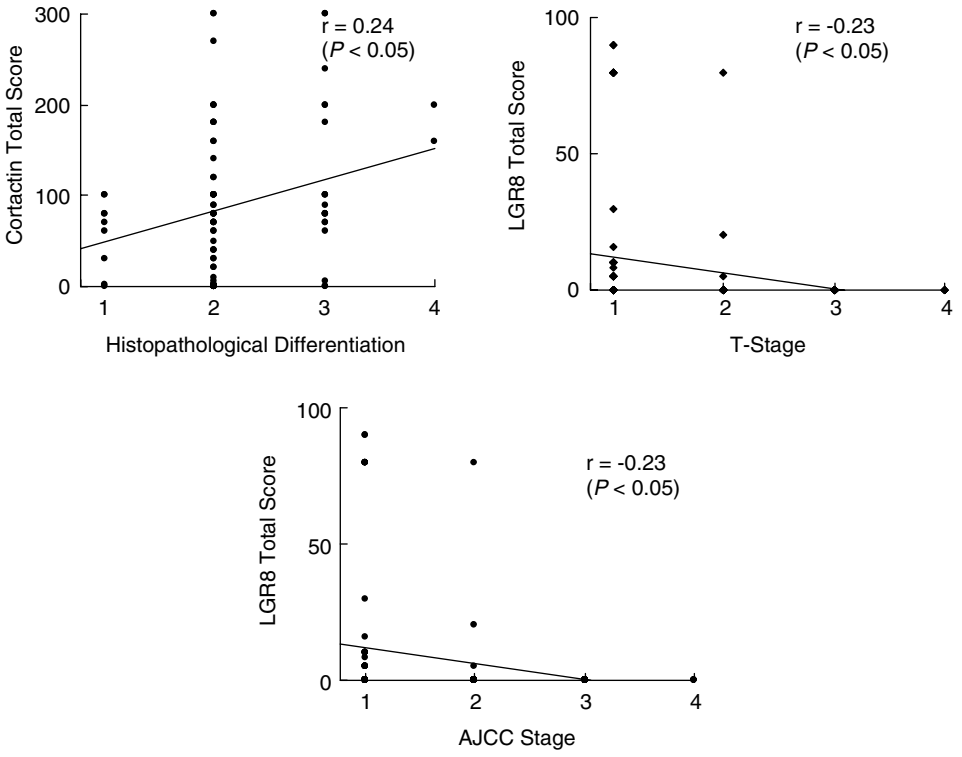

Fig. 2. Significant correlation between clinicopathological data and immunostaining scores of LGR8 and cortactin in hepatocellular carcinoma.

Results

Immunostaining Scores of LGR8 Correlate with T and AJCC Clinical Stages of Hepatocellular Carcinoma

Immunostaining scores for the six biomarkers in the HCC cases analyzed are presented in Table 1 and representative samples are illustrated in Fig. 1.

Among the 93 HCC cases, LGR8 immunostaining scores were significantly higher (3.48 ± 1.35, $P < 0.05$) (Table 1) than in the staining of the normal liver tissue. Among these tumors, 9 (10%) were well differentiated, 62 (67%) were moderately differentiated, 20 (21%) were poorly differentiated and 2 (2%) were anaplastic differentiation. Additional information, including TNM and AJCC clinical staging distributions, is presented in Table 2. Using the Pearson Product Method Correlation test, higher immunostaining scores of LGR8 showed a negative correlation with T stage and AJCC clinical stage, but not with N and M stages (Table 2 and Fig. 2).

Correlation of Immunostaining Scores of LGR8 with Upregulation of MMP-9 May Provide a Possible Mechanism in Cellular Invasiveness of Hepatocellular Carcinoma

Cellular pathways involving the matrix metalloproteinases (MMP) and tissue inhibitors of MMP (TIMP) are important in normal and abnormal physiological actions of cellular migration and invasion (Fig. 3). In our study, higher immunostaining scores of LGR8 showed a positive correlation with immunostaining scores of MMP-9 ($P < 0.05$; Fig. 4) which may provide a possible mechanism in cellular invasiveness of HCC. In addition, some studies have suggested that high circulating relaxin levels indirectly

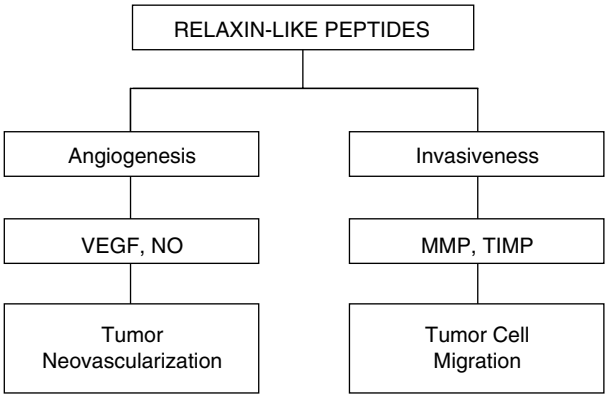

Fig. 3. Relaxin indirectly affects multiple steps in tumor progression and is a regulator of VEGF, NO, MMP and TIMP. Tumor cell migration and neovascularization are dependent on these factors.

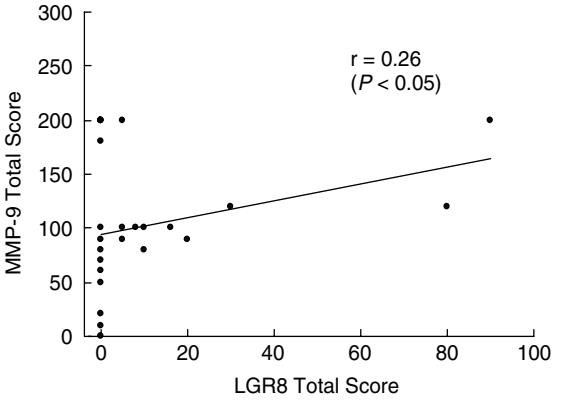

Fig. 4. Significant positive correlation between LGR8 total score and MMP-9 total score in hepatocellular carcinoma Survival rate were analyzed using the Kaplan-Meier survival test.

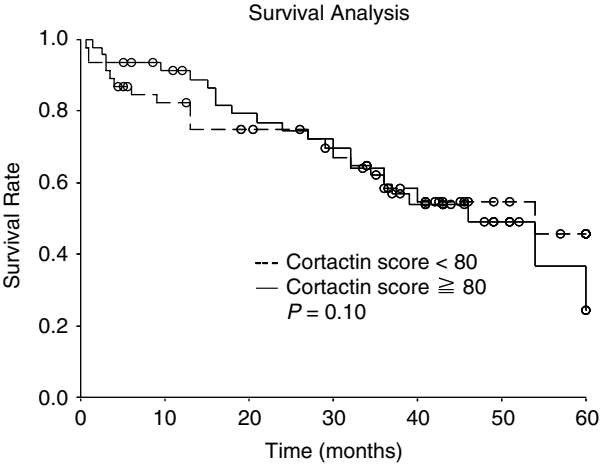

Fig. 5. Survival rates analyzed using the Kaplan-Meier survival test.

stimulate the formation of vasculature for tumor cell growth and invasion through the upregulation of VEGF in breast and prostate cancers (10), but in our study, higher immunostaining scores of LGR8 did not show a correlation with immunostaining scores of VEGF in HCC.

Immunostaining Scores of Cortactin Correlate with Histological Grades of Hepatocellular Carcinoma

Fascin-1 and cortactin are two important components among actin cross-linking proteins. Fascin-1 is markedly upregulated in several different types of tumors including breast, skin, lung, colon, brain, ovary, urinary bladder and gastric malignancies. And cortactin expression is frequently amplified in breast, head/neck carcinomas and gastric adenocarcinomas. In our study, cortactin immunostaining scores were significantly higher (84.88 ± 8.30, $P < 0.05$) (Table 1) than in the staining of the normal liver tissue. Using the Pearson Product Method Correlation test, higher immunostaining scores of cortactin showed a positive correlation with histological grading, but not AJCC clinical, and T, N or M stages. ($P < 0.05$; Table 2; Fig. 2). However, the immunostaining scores of fascin-1 in HCC were neither significantly higher than that of the normal liver tissue nor showed correlation with clinicopathologic parameters.

Relationship of Cortactin Expression to Survival Time

Consisting of 67 males and 26 females, the 93 patients with 5-year follow-up were divided into two groups on the basis of median cortactin immunostaining scores. The number of deaths in five years and the median survival time among the HCC patients with higher cortactin expression (n = 47, immunostaining scores ≧ 80) were 22 deaths and 46 months and those with lower cortactin expression (n = 46, immunostaining scores < 80) were 19 deaths and 54 months. Using the immunostaining scores as variables, higher cortactin immunostaining scores were not significantly associated with higher mortality in HCC ($P > 0.05$; Fig. 5).

Discussion

In many studies, multiple factors, such as virus infection, genetic mutation, cell cycle regulator dysfunction and imbalances in growth factors, have been shown to induce the incidence and progression of HCC (7). However, details on factors determining early metastasis and rapid progression of HCC are still unknown, but it is known that one requirement is enhancement of cell motility. In fact, enhanced movement of cancer cells has been reported to correlate with greater metastatic potentials in animal models and poorer prognosis in human cancers (3).

Relaxin indirectly affects multiple steps on tumor progression and it is an established regulator of VEGF and MMPs. Viability and migratory behavior of tumor cells are dependent on these factors. The identification of the relaxin/INSL3 receptor LGR8 has been a major advancement in relaxin-like research. This landmark discovery has provided the structural basis to elucidate the action and regulation of relaxin receptor-derived signal cascades induced by a novel auto/paracrine relaxin-like system potentially affecting important steps in carcinogenesis (17). In our study, higher immunostaining scores of LGR8 showed a negative correlation with T and AJCC clinical stages.

Tumor growth is critically dependent on angiogenesis and some studies show that high circulating relaxin levels indirectly stimulate the formation of

vasculature for tumor cell growth and invasion through upregulation of VEGF in breast and prostate cancers (10). In liver tumor, mechanisms of VEGF-induced metastasis have also been demonstrated (18).

The role of MMP-2 and MMP-9 has been extensively studied in the last few years and it has been shown that MMP-2 and MMP-9 are necessary for cancer cell attachment to distant sites (19). Upregulation of MMP-2, MMP-9 and MMP-14 by relaxin may provide one mechanism for the indirect role of relaxin in cellular invasiveness. These factors are associated with metastatic potential facilitating tumor cell migration across basement membrane (29). In our study, immunostaining scores of MMP-9, not of MMP-2, were significantly higher than in the staining of normal liver tissue. But both MMP-9 and MMP-2 in HCC were not associated with clinicopathological factors related to aggressive disease. In addition, higher immunostaining scores of LGR8 showed a positive correlation with immunostaining scores of MMP-9 supporting that the LGR8 and MMP-9 cascades seemed to play a role in early tumor cell migration in hepatocellular carcinoma.

Fascin-1, a 55-kDa globular protein, plays important roles in the formation of cellular protrusions, migration and extracellular matrix adhesion (1). Cortactin, a p80/85 protein, regulates the actin cytoskeleton through its involvement in cell motility, adhesion, polarization, contraction and others (35, 36). In a recent study, it was shown that overexpression of cortactin may play a role in the metastasis of HCC by influencing cell motility and intrahepatic metastasis in cell lines (4). In our study, overexpression of cortactin, but not fascin-1, was significantly correlated with tumor differentiation in human HCC tissues, and this observation also supports the role of cortactin being closely associated with intrahepatic metastasis, but neither cortactin nor fascin-1 immunostaining scores showed significant correlation with clinicopathological parameters in HCC. To our knowledge, this is the first report to evaluate association of expression of LGR8, VEGF, MMP-2, MMP-9, cortactin and fascin-1 with tumor progression in hepatocellular carcinoma.

In conclusion, although the understanding of relaxin-like actions in tumor biology is still in its early stages and there are more questions than answers. In our study, our data support the cascades that overexpression of LGR8 with upregulation of MMP-9 may play a role in the invasiveness of HCC by influencing cell migration in early AJCC clinical stage, but expression of VEGF immunostaining scores does not seem to be upregulated by LGR8 in tumor neovascularization. And finally, cortactin also plays a role in the migration or invasion of HCC; cortactin is especially closely associated with intrahepatic metastasis in human HCC although the precise mechanism remains to be elucidated.

Acknowledgments

This study was supported by a grant from Tri-Service General Hospital, TSGH-C98-55, Taiwan, R.O.C.

References

1. Adams, J.C. Fascin protrusions in cell interactions. *Trends Cardiovasc. Med.* 14: 221-226, 2004.
2. Allgaier, H.P., Deibert, P., Olschewski, M., Spamer, C., Blum, U., Gerok, W. and Blum, H.E. Survival benefit of patients with inoperable hepatocellular carcinoma treated by a combination of transarterial chemoembolization and percutaneous ethanol injection—a single-center analysis including 132 patients. *Int. J. Cancer* 79: 601-605, 1998.
3. Bauknecht, T., Birmelin, A. and Kommoss, F. Clinical significance of oncogenes and growth factors in ovarian carcinomas. *J. Steroid Biochem. Mol. Biol.* 37: 855-862, 1990.
4. Chuma, M., Sakamoto, M., Yasuda, J., Fujii, G., Nakanishi, K., Tsuchiya, A., Ohta, T., Asaka, M. and Hirohashi, S. Overexpression of cortactin is involved in motility and metastasis of hepatocellular carcinoma. *J. Hepatol.* 41: 629-636, 2004.
5. Goncharuk, V.N., Ross, J.S. and Carlson, J.A. Actin-binding protein fascin expression in skin neoplasia. *J. Cutan. Pathol.* 29: 430-438, 2002.
6. Grothey, A., Hashizume, R., Sahin, A.A. and McCrea, P.D. Fascin, an actin-binding protein associated with cell motility, is upregulated in hormone receptor negative breast cancer. *Brit. J. Cancer* 83: 870-873, 2000.
7. Hamilton, S.R. and Aaltonen, L.A. WHO classification of tumors of the digestive system. Lyon: *IRAC*. 159-180, 2000.
8. Hashimoto, Y., Skacel, M. and Adams, J.C. Roles of fascin in human carcinoma motility and signaling: prospects for a novel biomarker? *Int. J. Biochem. Cell Biol.* 37: 1787-1804, 2005.
9. Hisaw, F.L. Experimental relaxation of the pubic ligament of the guinea pig. *Proc. Soc. Exp. Biol. Med.* 23: 661-663, 1926.
10. Hombach-Klonisch, S., Buchmann, J., Sarun, S., Fischer, B. and Klonisch, T. Relaxin-like factor (RLF) is differentially expressed in the normal and neoplastic human mammary gland. *Cancer* 89: 2161-2168, 2000.
11. Hombach-Klonisch, S., Hoang-Vu, C., Kehlen, A., Hinze, R., Holzhausen, H.J., Weber, E., Fischer, B., Dralle, H. and Klonisch, T. INSL-3 is expressed in human hyperplastic and neoplastic thyrocytes. *Int. J. Oncol.* 22: 993-1001, 2003.
12. Hsu, S.Y., Nakabayashi, K., Nishi, S., Kumagai, J., Kudo, M., Sherwood, O.D. and Hsueh, A.J. Activation of orphan receptors by the hormone relaxin. *Science* 25: 671-674, 2002.
13. Hu, W., McCrea, P.D., Deavers, M., Kavanagh, J.J., Kudelka, A.P. and Verschraegen, C.F. Increased expression of fascin, motility associated protein, in cell cultures derived from ovarian cancer and in borderline and carcinomatous ovarian tumors. *Clin. Exp. Metastasis* 18: 83-88, 2000.
14. Ivell, R. and Einspanier, A. Relaxin peptides are new global players. *Trends Edocrinol. Metab.* 13: 343-348, 2002.
15. Jawhari, A.U., Buda, A., Jenkins, M., Shehzad, K., Sarraf, C., Noda, M., Farthing, M.J., Pignatelli, M. and Adams, J.C. Fascin, an actin-bundling protein, modulates colonic epithelial cell invasiveness and differentiation *in vitro*. *Am. J. Pathol.* 162: 69-80, 2003.
16. Klonisch, T., Ivell, R., Balvers, M., Kliesch, S., Fischer, B., Bergmann, M. and Steger, K. Expression of relaxin-like factor is down-regulated in human testicular Leydig cell neoplasia. *Mol.*

Hum. Reprod. 5: 104-108, 1999.
17. Kumagai, J., Hsu, S.Y., Matsumi, H., Roh, J.S., Fu, P., Wade, J.D., Bathgate, R.A. and Hsueh, A.J. INSL3/Leydig insulin-like peptide activates the LGR8 receptor important in testis descent. *J. Biol. Chem.* 30: 31283-31286, 2002.
18. Mise, M., Arii, S., Higashituji, H., Furutani, M., Niwano, M., Harada, T., Ishigami, S., Toda, Y., Nakayama, H., Fukumoto, M., Fujita, J. and Imamura, M. Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. *Hepatology* 23: 455-464, 1996.
19. Musso, O., Théret, N., Campion, J.P., Turlin, B., Milani, S., Grappone, C. and Clément, B. In situ detection of matrix metalloproteinase-2 (MMP2) and the metalloproteinase inhibitor TIMP2 transcripts in human primary hepatocellular carcinoma and in liver metastasis. *J. Hepatol.* 26: 593-605, 1997.
20. Ormandy, C.J., Musgrove, E.A., Hui, R., Daly, R.J. and Sutherland, R.L. Cyclin D1, EMS1 and 11q13 amplification in breast cancer. *Breast Cancer Res. Treat.* 78: 323-335, 2003.
21. Parkin, D.M., Pisani, P. and Ferlay, J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int. J. Cancer* 80: 827-841, 1990.
22. Parsell, D.A., Mak, J.Y., Amento, E.P. and Unemori, E.N. Relaxin binds to and elicits a response from cells of the human monocytic cell line, THP-1. *J. Biol. Chem.* 271: 27936-27941, 1996.
23. Pelosi, G., Pastorino, U., Pasini, F., Maissoneuve, P., Fraggetta, F., Iannucci, A., Sonzogni, A., De Manzoni, G., Terzi, A., Durante, E., Bresaola, E., Pezzella, F. and Viale, G. Independent prognostic value of fascin immunoreactivity in Stage I non-small cell lung cancer. *Brit. J. Cancer* 88: 537-547, 2003.
24. Roma, A.A. and Prayson, R.A. Fascin expression in 90 patients with glioblastoma multiforme. *Ann. Diagn. Pathol.* 9: 307-311, 2005.
25. Schafer, D.F. and Sorrell, M.F. Hepatocellular carcinoma. *Lancet* 353: 1253-1257, 1999.
26. Schuuring, E. The involvement of the chromosome 11q13 region in human malignancies: cyclin D1 and EMS1 are two new candidate oncogenes–a review. *Gene* 159: 83-96, 1995.
27. Silvertown, J.D., Summerlee, A.J. and Klonisch, T. Relaxin-like peptides in cancer. *Int. J. Cancer* 107: 513-519, 2003.
28. Stemmermann, G.N., Mesiona, W., Greenwood, F.C. and Bryant-Greenwood, G.D. Immunocytochemical identification of a relaxin-like protein in gastrointestinal epithelium and carcinoma: a preliminary report. *J. Endocrinol.* 140: 321-325, 1994.
29. Sugiura, Y., Shimada, H., Seeger, R.C., Laug, W.E. and DeClerck, Y.A. Matrix metalloproteinases-2 and -9 are expressed in human neuroblastoma: contribution of stromal cells to their production and correlation with metastasis. *Cancer Res.* 58: 2209-2216, 1998.
30. Tong, G.X., Yee, H., Chiriboga, L., Hernandes, O. and Waisman, J. Fascin-1 expression in papillary and invasive urothelial carcinomas of the urinary bladder. *Hum. Pathol.* 36: 741-746, 2005.
31. Tsai, W.C., Jin, J.S., Chang, W.K., Chan, D.C., Yeh, M.K., Cherng, S.C., Lin, L.F., Sheu, L.F. and Chao, Y.C. Association of cortactin and fascin-1 expression in gastric adenocarcinoma: correlation with clinocopathological parameters. *Dis. Markers* 55: 955-962, 2007.
32. Unemori, E.N., Erikson, M.E., Rocco, S.E., Sutherland, K.M., Parsell, D.A., Mak, J. and Grove, B.H. Relaxin stimulates expression of vascular endothelial growth factor in normal human endometrial cells *in vitro* and is associated with menometrorrhagia in women. *Hum. Reprod.* 14: 800-806, 1999.
33. Unemori, E.N., Lewis, M., Constant, J., Arnold, G., Grove, B.H., Normand, J., Deshpande, U., Salles, A., Pickford, L.B., Erikson, M.E., Hunt, T.K. and Huang, X. Relaxin induces vascular endothelial growth factor expression and angiogenesis selectively at wound sites. *Wound Repair Regen.* 8: 361-370, 2000.
34. Van Rossum, A.G., Moolenaar, W.H. and Schuuring, E. Cortactin affects cell migration by regulating intercellular adhesion and cell spreading. *Exp. Cell Res.* 312: 1658-1670, 2006.
35. Van Rossum, A.G., Gibcus, J., van der Wal, J. and Schuuring, E. Cortactin overexpression results in sustained epidermal growth factor receptor signaling by preventing ligand-induced receptor degradation in human carcinoma cells. *Breast Cancer Res.* 7: 235-237, 2005.
36. Weed, S.A. and Parsons, J.T. Cortactin: coupling membrane dynamics to cortical actin assembly. *Oncogene* 20: 6418-6434, 2001.