RESEARCH COMMUNICATION

High Expression of CXCR7 Combined with Alpha Fetoprotein in Hepatocellular Carcinoma Correlates with Extra-hepatic Metastasis to Lung after Hepatectomy

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Abstract

The lung is the most frequent metastatic site of hepatocellular carcinoma (HCC), negatively impacting on survival rates. In this study, we evaluated the prognostic role of the chemokine receptor CXCR7 in lung metastasis of HCC after hepatectomy, using immunohistochemical detection on tissue microarrays of HCCs, with and without lung metastasis. Using three categories based on staining characteristics, patients with high CXCR7 expression demonstrated a shorter time to development of lung metastasis compared with patients with low CXCR7 expression (log-rank test) with no effet on overall survival. Analysis of tissue adjacent to tumor showed patients with microvascular invasion to have higher CXCR7. Stratification based on alpha fetoprotein level >20 ng/ml also showed high expression of CXCR7 to be a strong independent prognostic factor. These findings suggest that high expression of CXCR7 in HCCs with elevated alpha fetoprotein levels correlates with metastasis to lung and poor survival after hepatectomy, indicating potential use as a prognostic factor.

Keywords: Chemokine receptor - CXCR7 - metastasis - hepatocellular carcinoma - alpha fetoprotein

Introduction

Metastasis correlates with poor survival rate and long-term prognosis of patients with hepatocellular carcinoma (HCC). Even if accepted the hepatic resection, some of patients with HCC inevitably died because of recurrence and metastasis. Prognosis remains poor for extra-hepatic metastasis and treatment for it seems to be required to further improve survival. Outcomes after resection in patients with HCC treated in centers in the United States, France, and Japan showed 5-year survival rates only 31%~41% in these followed-up patients accepted the hepatectomy, because of recurrence and metastasis (Esnaola et al., 2003). Recent research suggests that early detection of recurrent tumors is important. Re-resection correlates with better post-recurrent survival rates (Chen et al., 2004). Surgical resection for pulmonary metastasis from HCC might be beneficial for patients whose HCC was controlled by surgery and the number of lesions was lower than 3 (Lee et al., 2010). Therefore, identification of prognostic factors of metastasis after hepatectomy might help plan the relative special follow-up schedule, find and intervene the metastasis early, which may makes these patients acquire the survival benifits.

The most frequent metastatic site of HCC is lung (Katyal et al., 2000; Natsuizaka et al., 2005). Previous work suggested the process of metastasis to lung was not random but organ-specific based on the finding that extractions from lung but not from liver, renal, and heart had the prominent chemoattractive effects to the HCC cell line with strong metastatic potential (Ji et al., 2003). In addition, many patients with HCC occur the lung metastasis whereas not have the detactable intra-hepatic recurrence, which suggests the heterogeneity of tumor cells may have the potential effects on organ-specific metastasis of HCC. Identification of prognostic factors linked to the lung metastasis of HCC after hepatic resection may help to promote the survival of these patients. However, report about prognostic factors to correlate with the organ-specific metastasis of HCC after hepatic resection is limit.

Chemokine receptors, belonging to G-protein coupled seven-transmembrane receptors, have been demonstrated to play the critical roles in organ-specific metastasis of tumors (Ben-Baruch 2008). CXCR7 , once named orphan receptor because of unknown ligand, is now recognized as a new chemokine receptor. CXCR7 was showed to have the definitive ligand stromal cell-derived factor-1 (SDF-1) and truncated I-TAC (CXCL11) (Balabanian et al., 2005; Burns et al., 2006), and can participate in the nonclassic pathway by activate mitogen-activated protein kinases through β-arrestins (Rajagopal et al., 2010). CXCR7 expression has been demonstrated to increase as the tumors become more aggressive in model

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of prostate tumors. Alterations in CXCR7 expression are associated with enhanced adhesive and invasive activities of prostate cancer cells (Wang et al., 2008). Also, higher expression of chemokine receptor CXCR7 was found to be linked to early and metastatic recurrence in pathological stage I nonsmall cell lung cancer (Iwakiri et al., 2009). Just recently, research showed the potential roles of over-expression of CXCR7 in invasion and growth of HCC, which can be affected by vascular endothelial growth factor stimulation (Zheng et al., 2010). Besides, in osteoarthritis model, over-expressed CXCR7 in chondrocyte cells can induce the increased expressions of osteopontin, interleukin-8, matrix metalloproteinases-2, and vascular endothelial growth factor, (Jones et al., 2006) which suggests the potential roles of CXCR7 in metastasis of HCC because of the close correlations between these genes and metastasis of HCC.

Here, we evaluated the prognostic role of CXCR7 in lung metastasis and survival of human HCC after hepatic resection. Tissue microarray (TMA) was constructed on the basis of the tumor tissues and tissue adjacent to tumor (TAT) in followed-up patients after hepaectomy. Thirteen characters including CXCR7 were analyzed the potential predictive role for lung metastasis after hepatic resection.

Materials and Methods

Patients

One hundred and sixteen patients were retrieved from the prospectively designed database. Informed consents were obtained from the patients. These patients underwent hepatectomy by the same surgical team from January 2000 to May 2004. The hepatectomy of HCC was carried out as described previously (Sun et al., 2007). Simply to say, The indications for hepatectomy were that the main tumor was technically resectable, no cancerous thrombi were present in the main trunk of the portal vein, and no distant metastasis was found. These patients were proven HCC pathologically after resection. Hematoxylin and eosin stained paraffin tissue sections were reviewed by two pathologists according to the WHO histomorphologic criteria.

There were 103 men and 13 women with a mean age of 51.0 ± 11.4 years (range, 18-78 years). Ninety-four patients were positive for the hepatitis B surface antigen (HBsAg). All patients were classified as Child A.

Follow-up

Regular follow-up examinations in our clinic are as follows: serum alpha fetoprotein (AFP) assay and liver ultrasonography every 3 months during the first year, then every 6 months; and magnetic resonance imaging (MRI) or liver computed tomography (CT) scanning at 1 and 6 months, then every 6 months. Chest X-ray is regularly used to examine the lung metastasis. If lung metastasis was suspected, chest enhanced CT scan was performed immediately. Lung metastasis was demonstrated by biopsy through endoscopy or pathology after lung partial resection. Until May 2009, fifty-eight patients were found lung metastases. Ten patients with resectable lung metastasis accepted the lung partial resection.

TMA and immunohistochemistry

First, hematoxylin and eosin-stained slides were screened for optimal tumor content and TATs with a distance of 2 cm from the tumor. Then TMA was constructed in accordance with standard procedures (Simon et al., 2004) based on 116 tumor tissues and 47 TATs. Two cores were taken from each formalin-fixed, paraffin-embedded HCC samples by using punch cores that measured 1.0mm in greatest dimension from the center of tumor foci and TAT. A 2-step method of immunohistochemistry including heat-induced antigen-retrieval procedures was performed to detect the CXCR7 and CXCR4. Sections were incubated overnight at 4°C with primary antibody and washed. Then the immunohistochemistry detection was carried out in avidin-biotin complex method (ABC, Vector Laboratories, Burlingame, CA). Rabbit anti-human CXCR7 IgG (Novus Biologicals, Littleton, CO) at 1:200 and rabbit anti-human CXCR4 IgG (Santa Cruz Biotech, Santa Cruz, CA) at 1:100 were used as primary antibodies in detection. Detection without primary antibody was considered negative control.

Scoring and categories of expression of CXCR7 and CXCR4

The immunoassay was defined by the staining intensity (0-3) and the percentage of positive tumor cells as described previously (Lugli et al., 2005). Simply to say, the staining results were categorized into four groups. Tumors without any staining were considered negative. Tumors with 1+ staining intensity in <80% of cells and 2+ intensity in <30% of cells were considered weakly positive. Tumors with 1+ staining intensity in >80% of cells, 2+ intensity in 30% to 79% or 3+ intensity in <30% were considered moderately positive. Tumors with 2+ intensity in >80% or 3+ intensity in >30% of cells were considered strongly positive. In this study, we recognized these four groups of staining as A, B, C, and D, respectively. Two pathologists observed the results independently.

Three categories of patients according to staining scoring were set up as follows:

a) Category 1: Staining A, B, and C were grouped as CXCR7^{low}, and staining D was grouped as CXCR7^{high};

b) Category 2: Staining A was grouped as CXCR7^{low}. Staining B and C were grouped as CXCR7[medium], and staining D was grouped as CXCR7^{high};

c) Category 3: Only detectable staining of CXCR7 expression were grouped. Staining B and C were grouped as CXCR7^{low}, and staining D was grouped as CXCR7^{high}.

Methods of categories for CXCR4 were as same as for CXCR7.

Statistical analysis

Time-to-lung metastasis and overall survival (OS) were observed in survival analysis, Kaplan-Meier method was used to describe the survival curves, and log-rank test was used to compare survival distributions between groups. Breslow test also was used when survival curves indicated the more difference during the early follow-up period. Time-to-lung metastasis was calculated from the date of hepatectomy to the date of lung metastasis with definite clinical diagnosis. OS was calculated from the

date of hepatectomy to the date of death regardless of cause. The lost patients in the follow-up and the patients who were still not occurred the desired event at the end of this study were recognized as censored cases. Log-rank method was also used for prognostic factors screen in univariable analysis. Cox-regression model was used to identify the prognostic factors related to time-to-lung metastasis and OS.

Thirteen potential prognostic factors were all categorized variables. These included 4 clinical factors (age <= 60 years or >60 years, gender, HBsAg status, and serum AFP level <= 20 ng/ml or >20 ng/ml), and 9 pathologic factors (cirrhotic or noncirrhotic, tumor size<=5cm or >5cm, single or multiple tumor nodules, well or poorly encapsulated tumor, presence or absence of microvascular invasion, portal lymphatic status, Edmondson Grade 1/2 or Grade 3/4, CXCR7 staining, and CXCR4 staining). The relationship between gene expression and the clinical and pathological parameters was determined using Pearson Chi-Square test and Fisher's exact test where appropriate. All statistical analyses were completed by using SPSS15.0 software (SPSS Inc., Chicago, IL). A 2-sided P<0.05 was considered statistically significant.

Results

Clinicopathologic data

CXCR7 was stained mainly in the membrane and cytoplasm of HCC (Figure 1). According to the scoring methods, stainings of A, B, C, and D of CXCR7 expression were observed in 20, 11, 34, and 51 patients, respectively. In patients with lung metastasis, most of them died in five years after operation, including 1 patient who died of stroke, 3 patients who died of liver failure, 1 patient who died of brain metastasis, and 1patient who died of renal failure.

In category 1, CXCR7^{high} and CXCR7^{low} were observed in 51 patients and 65 patients, respectively. There was no statistically difference between CXCR7 expression and clinicopathologic data except for tumor size (Table 1). In TAT analysis of clinicopathologic data, patients with age<=60 years (P=0.041) and with microvascular invasion (P=0.002) expressed higher CXCR7. On the other hand, we found subgroup with AFP >20ng/ml (P=0.001) and with poorly encapsulated tumor (P=0.025) expressed lower CXCR4.

In category 2, CXCR7^{high}, CXCR7[medium], and CXCR7^{low} were observed in 51, 45 and 20 patients, respectively. There was no significant difference in clinicopathologic data of three groups of patients. In TAT analysis of clinicopathologic data, patients with age<=60 years (P=0.015) and with microvascular invasion (P=0.003) expressed higher CXCR7.

In category 3: CXCR7^{high} and CXCR7^{low} were observed in 51 patients and 45 patients, respectively. There was no significant difference between CXCR7 expression and clinicopathologic data except for AFP level (P=0.03). Patients with AFP >20 ng/ml expressed higher CXCR7. In TAT analysis of clinicopathologic data (n=41), patients with microvascular invasion (P=0.002) expressed higher CXCR7.
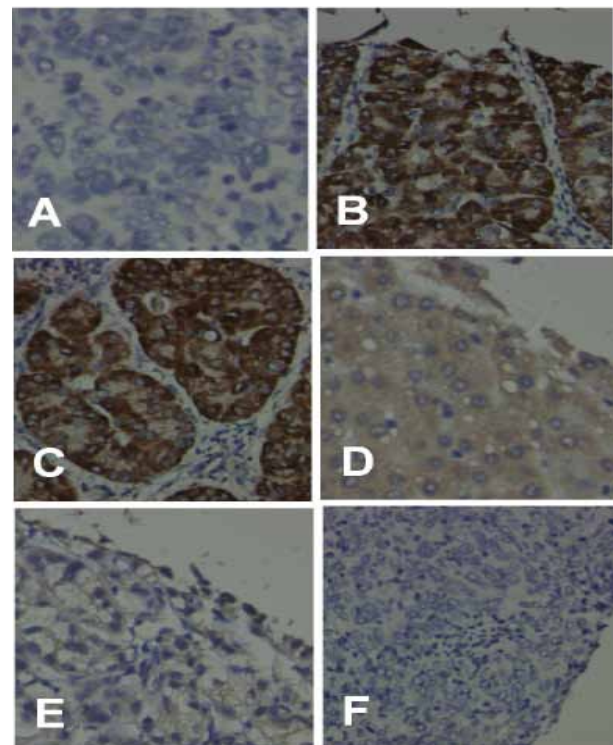
Figure 1. Immuno-staining Character and Staining Intensity of CXCR7 in HCC. A, HCC cells were round or oval with increased karyoplasmic ratio and localized or scattered; B, The immunopositivity for CXCR7 was mainly observed in the membrane or the cytoplasm of HCC cells; C-F, Four groups of staining intensity from strong positive to negative were showed respectively. Original magnification, ×400 (A-F)
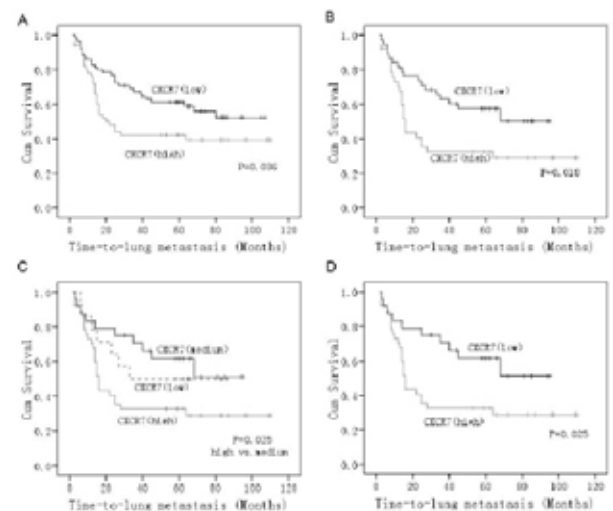
Figure 2. High Expression of CXCR7 Correlates with Short Time to Metastasize to Lung in HCC Patients after Hepatectomy. A, Compared in category 1; B, Stratification analysis based on AFP level in category 1; C, Stratification analysis based on AFP level in category 2; D, Stratification analysis based on AFP level in category 3 (Kaplan-Meier, log-rank test)

Analysis of category 1

a) Patients with high CXCR7 expression in tumor metastasized to lung in shorter time : In group CXCR7^{low}, the cumulative 1-year, 3-year, 5-year, and 7-year rates of time-to-lung metastasis were 77%, 61%, 57%, and 53%, respectively, whereas in group CXCR7^{high}, the cumulative

Table 1. Clinicopathologic Factors and CXCR7 Expression in Category 1

Clinicopathologic Data		No. of Patients		
		CXCR7[low] (n=65)	CXCR7[high] (n=51)	P
Age, y	<=60	54	42	0.92
	>60	11	9	
Gender	Male	59	44	0.45
	Female	6	7	
HbsAg	Negative	12	10	0.88
	Positive	53	41	
Cirrhosis	Absent	48	42	0.28
	Present	17	9	
AFP, ng/ml	<=20	27	13	0.07
	>20	38	38	
Tumor Size	<=5cm	28	13	0.05
	>5cm	37	38	
No.of Tumor Nodules	Single	51	40	0.1
	Multiple	14	11	
Tumor Capsule	Well Capsuled	39	33	0.60
	Poorly Capsuled	26	18	
Microvascular Invasion	Negative	48	31	0.13
	Positive	17	20	
Edmondson Grade	Grade(1/2)	39	34	0.46
	Grade(3/4)	26	17	
Portal Lymphatic Status*	No	62	46	0.3
	Yes	3	5	

HCC, hepatocellular carcinoma; N, number; *, Fisher's exact test

1-year, 3-year, 5-year, and 7-year rates were 48%, 42%, 39%, and 39%, respectively. Time metastasized to lung for group CXCR7^{high} was shorter than time for group CXCR7^{low} (P=0.036, log-rank test). The curve of time-to-lung metastasis was shown in Figure 2A. The median time developing into lung metastasis for group CXCR7^{high} was significantly shorter than that of group CXCR7^{low} (23.3 months versus (vs) 96 months).

In group CXCR7^{low}, the cumulative 1-year, 3-year, 5-year, and 7-year OS rates were 81%, 62%, 57%, and 57%, respectively, whereas in group CXCR7^{high}, the cumulative 1-year, 3-year, 5-year, and 7-year OS rates were 58%, 47%, 45%, and 45%, respectively. There was no statistical difference in OS between group CXCR7^{high} and group CXCR7^{low} (P=0.062, log-rank test). However, early stage of follow-up indicated the significant difference (P=0.022, Breslow test). The median OS of group CXCR7^{high} was significantly shorter than that of group CXCR7^{low} (35.3 months vs 96 months).

b) CXCR7 as a potential prognostic factor for lung metastasis of HCC : Univariate analysis indicated that AFP (P=0.026), microvascular invasion (P=0.000), portal lymphatic status (P=0.003), Edmondson Grade (P=0.021), and CXCR7 (P=0.036) were potential prognostic factors for lung metastasis. Further Cox analysis showed that microvascular invasion (RR, 3.0; 95% confidence interval (95% CI), 1.7-5.2 (P=0.000)) and Edmondson Grade (RR, 2.3; 95% CI, 1.3-3.9 (P=0.021)) were identified as the independent prognostic factors in lung metastasis of HCC. CXCR7 (RR, 1.6; 95% CI, 1.0-2.7 (P=0.077)) showed to be potential prognostic factor, and group CXCR7^{high} had 1.6 times chances to occur the lung metastasis than group CXCR7^{low}.

c) CXCR4 staining was seemly a protective prognostic factor for development into lung metastasis : Because CXCR4 shares same ligand SDF-1 with CXCR7 and has the critical roles in metastases of many tumors, potential prognostic role of CXCR4 in lung metastasis of HCC was also analyzed. In group CXCR4^{high}, the cumulative 1-year, 3-year, 5-year, and 7-year rates of time-to-lung metastasis were 64%, 52%, 44%, and 44%, respectively, whereas in group CXCR4^{low}, the cumulative 1-year, 3-year, 5-year, and 7-year rates were 49%, 39%, 36%, and 30%, respectively. The median survival time of group CXCR4^{high} was significantly longer than that of group CXCR4^{low} (108 months vs 44.5 months), although there were no significant differences in time-to-lung metastasis (P=0.264, log rank test) and OS (P=0.626, log-rank test).

d) Stratum 1 based on AFP level: CXCR7 staining showed strong prognostic effect for lung metastasis of patients with AFP >20ng/ml.

Because of the potential prognostic effects of CXCR7 and AFP for time-to-lung metastasis, combined predictive probability of CXCR7 and AFP were analyzed. In 76 HCCs with AFP(+), or AFP level > 20ng/ml, subgroup CXCR7^{high} metastasized to lung in shorter time compared with subgroup CXCR7^{low} (P=0.018, log-rank test) (Figure 2B). The cumulative 1-year, 3-year, 5-year, and 7-year rates of time-to-lung metastasis were higher in group AFP(+)CXCR7^{low} than in group AFP(+)CXCR7^{high} (74%, 57%, 53%, and 53% vs 41%, 33%, 29%, and 29%, respectively). The median time developing into lung metastasis of group AFP(+)CXCR7^{high} was significantly shorter than that of group AFP(+)CXCR7^{low} (20.6 months vs 84 months). Group CXCR7^{low} showed the higher cumulative 1-year, 3-year, 5-year, and 7-year OS rates than group CXCR7^{high} (76%, 53%, 50%, and 50% vs 58%, 47%, 45%, and 45%, respectively), although there was no significant difference in OS between group CXCR7^{high} and group CXCR7^{low} (P=0.125). The median OS of group CXCR7^{high} was only half of group CXCR7^{low} (28.0 months vs 59.7 months).

Univariate analysis showed that CXCR7 (P=0.018), microvascular invasion (P=0.001), portal lymphatic status (P=0.008) were potential prognostic factors for lung metastasis in patients with AFP(+). CXCR7 (RR, 2.23; 95% CI, 1.20-4.16 (P=0.012)) and microvascular invasion (RR, 2.86; 95% CI, 1.54-5.32 (P=0.001)) were 2 independent prognostic factors in Cox analysis. Subgroup CXCR7^{high} had more than twice likelihood to metastasize to lung than subgroup CXCR7^{low} in patients with AFP>20ng/ml.

In addition, we did not observe the statistical difference of time-to-lung metastasis between subgroup CXCR4^{high} and subgroup CXCR4^{low} in patients with AFP(+) (P>0.05).

e) Stratum 2: prognostic role of CXCR7 staining for lung metastasis was seemly enhanced when combined with CXCR4 expression.

Because time to develop into lung metastasis is shorter when patients expressed CXCR4 lowly, combined predictive probability of CXCR7 and CXCR4 were analyzed. In 72 HCCs with CXCR4^{low}, there was not significant difference in time-to-lung metastasis between the subgroup CXCR7^{high} and subgroup CXCR7^{low}

(P=0.122, log-rank test). However, the median time-to-lung metastasis of group CXCR4^low^CXCR7^high^ was significantly lower than group CXCR4^low^CXCR7^low^ (21.4 months vs 82.3 months), and also lower than group CXCR7^high^. Also, the cumulative 1-year, 3-year, 5-year, and 7-year rates of time-to-lung metastasis of CXCR4^low^CXCR7^high^ were 43%, 36%, 36%, and 36%, respectively, which was lower than 70%, 58%, 54%, and 49% of group CXCR4^low^CXCR7^low^, and lower than those of group CXCR4^low^ at least during first 3 years followed-up. On the other hand, in 51 HCCs with CXCR7^high^, subgroup CXCR4^high^ metastasized to lung in longer time compared with subgroup CXCR4^low^ (P=0.023, log-rank test).

Analysis of category 2

a) Patients with high CXCR7 expression in tumor metastasized to lung in shorter time : In this category, staining A , or undetectable CXCR7 expression, was separated from group CXCR7^low^ of category 1.

Group CXCR7^high^ had the poor OS and was faster to occur lung metastasis than group CXCR7^medium^ (P=0.026 and P=0.007, respectively, log-rank test). Group CXCR7^medium^ had shorter time metastasized to lung than group CXCR7^low^ (P=0.027), although there was no significant difference in OS between group CXCR7^low^ and group CXCR7^medium^ (P>0.05, log-rank test).

Univariable analysis showed that AFP level (P=0.026), microvascular invasion (P=0.000), portal lymphatic status (P=0.003), Edmondson Grade (P=0.021), and CXCR7 (P=0.019) were potential prognostic factors for time-to-lung metastasis. Further, CXCR7 (medium vs high) (P=0.031), CXCR7^medium^ (RR, 0.47; 95% CI, 0.3-0.9 (P=0.016)), microvascular invasion (RR, 3.1; 95% CI, 1.8-5.5 (P=0.000)), and Edmondson Grade (RR, 2.3; 95% CI, 1.3-3.9 (P=0.003)) were identified as the independent prognostic factors for time-to-lung metastasis in Cox analysis.

b) Stratum based on AFP level : CXCR7 staining showed strong prognostic effect for lung metastasis of patients with AFP >20ng/ml.

In 76 HCCs with AFP(+), or AFP level > 20ng/ml, subgroup CXCR7^high^ metastasized to lung in shorter time compared with subgroup CXCR7^medium^ (P=0.025, log-rank test) (Figure 2C). Univariate analysis showed that microvascular invasion (P=0.001), portal lymphatic status (P=0.008) were potential prognostic factors for lung metastasis in patients with AFP(+) except for CXCR7. If CXCR7 was maintained in the model, CXCR7 (medium vs high, RR, 0.38; 95% CI, 0.18-0.81 (P=0.012)) and microvascular invasion (RR, 2.94; 95% CI, 1.57-5.51 (P=0.001)) were 2 independent prognostic factors. Subgroup CXCR7^high^ had more than twice likelihood to metastasize to lung than subgroup CXCR7^medium^ in patients with AFP>20ng/ml.

Analysis of category 3

a) Patients with high CXCR7 expression in tumor metastasized to lung in shorter time. : In this category, patients with staining A, or patients who can not be detected CXCR7 expression, were aparted from the observation. Among 96 patients with detectable CXCR7 expression, the cumulative 1-year, 3-year, 5-year, and 7-year rates of time-to-lung metastasis in group CXCR7^low^ were 87%, 68%, 65%, and 61%, respectively, whereas in group CXCR7^high^, the cumulative 1-year, 3-year, 5-year, and 7-year rates were 48%, 42%, 39%, and 39%, respectively. Time metastasized to lung for group CXCR7^high^ was shorter than time for group CXCR7^low^ (P=0.007, log-rank test). The median time developing into lung metastasis for group CXCR7^high^ was significantly shorter than that of group CXCR7^low^ (23.3 months vs 96 months).

In group CXCR7^high^, the cumulative 1-year, 3-year, 5-year, and 7-year OS rates were 58%, 47%, 45%, and 45%, respectively, which was lower than 89%, 68%, 60%, and 60% in group CXCR7^low^ (P=0.026), respectively. The median OS of group CXCR7^high^ was significantly shorter than that of group CXCR7^low^ (35.3 months vs 96 months).

Univariate analysis of potential prognostic factors for lung metstasis indicated that AFP level (P=0.003), microvascular invasion (P=0.004), portal lymphatic status (P=0.005), Edmondson Grade (P=0.033), and CXCR7 (P=0.007) were potential prognostic factors. Cox analysis further showed that CXCR7 (RR, 2.00; 95% CI, 1.08-3.70 (P=0.028)), microvascular invasion (RR, 2.45; 95% CI, 1.72-4.55 (P=0.004)), AFP level (RR, 2.24; 95% CI, 1.10-4.57 (P=0.026)), and Edmondson Grade(RR, 2.09; 95% CI, 1.15-3.78 (P=0.015)) were prognostic factors if CXCR7 was maintained in the model. Group CXCR7^high^ had twice potential to occur the lung metastasis than group CXCR7^low^.

b) Stratum based on AFP level: CXCR7 staining showed strong prognostic effect for lung metastasis in patients with AFP >20ng/ml.

In 62 HCCs with AFP(+), or AFP level > 20ng/ml, subgroup CXCR7^high^ metastasized to lung in shorter time compared with subgroup CXCR7^low^ (P=0.025, log-rank test) (Figure 2D). The cumulative 1-year, 3-year, 5-year, and 7-year rates of time-to-lung metastasis in group AFP(+)CXCR7^low^ were higher than in group AFP(+) CXCR7^high^ (79%, 61%, 55%, and 55% vs. 41%, 33%, 29%, and 29%, respectively). The median time developing into lung metastasis of group AFP(+)CXCR7^high^ was significantly shorter than that of group AFP(+)CXCR7^low^ (20.6 months vs 84 months). Group CXCR7^high^ showed the lower cumulative 1-year, 3-year, 5-year, and 7-year OS rates than group CXCR7^low^ (50%, 34%, 31%, and 31% vs 79%, 61%, 54%, and 54%, respectively). There was no statistical difference in OS between group CXCR7^high^ and group CXCR7^low^ (P=0.133, log-rank test). However, survival curve indicated the significant difference during the early stage of follow-up (P=0.038, Breslow test). The median OS of group CXCR7^high^ was only half of group CXCR7^low^ (26.0 months vs 58.0 months).

Univariate analysis showed that CXCR7 (P=0.025), microvascular invasion (P=0.052), portal lymphatic status (P=0.018) were potential prognostic factors for lung metastasis in patients with AFP(+). CXCR7 (RR, 2.43; 95% CI, 1.15-5.10 (P=0.020)) and microvascular invasion (RR, 2.09; 95% CI, 1.06-4.13 (P=0.034)) were 2 independent prognostic factors in Cox analysis.

Subgroup CXCR7^{high} had more than twice likelihood to metastasize to lung than subgroup CXCR7^{low} in patients with AFP>20ng/ml.

Discussion

Lung is the most frequent metastatic site of HCC and metastasis is one of the main hedges to promote the survival rate of patients. This study indicated that CXCR7, particularly combined with AFP level, was a valuable prognostic indicator for lung metastasis and poor prognosis of HCC after hepatectomy. In all 3 examined categories, group CXCR7^{high} showed the prominent short time developing lung metastasis and OS rates compared with group CXCR7^{low}, especially during the early stage of follow-up. Stratification based on AFP level indicated that high expression of CXCR7 was strong correlated with the lung metastasis and poor prognosis in patients with increased AFP level detected before hepatectomy. Reports about prognostic factors to predict the organ-specific metastasis of HCC are limit. Accumulation of chromosomal changes was found to be associated with metastatic behaviour of HCC, and LOH on 16q was the useful prognostic indicator for metastasis after curative resection of HCC (Nishida et al., 2002). HBsAg was reported the ability to predict extraheptic metastasis after hepatic resection in patients with large HCC (Sasaki et al., 2007). In this study, however, HBsAg was not shown to be correlated with time to metastasize to lung after hepatectomy. We think that one of possible reasons is patients we observed have different size tumors, and our observed end point was time to developing lung metastasis specifically but not other potential organs.

Further, in this study, stratification analysis indicated that elevated AFP level enhanced the prognostic roles of CXCR7 in time-to-lung metastasis and OS. It is well known that serum AFP is one of the biomarkers and prognostic factors of HCC. AFP was found to increase the accuracy of criteria with vascular invasion and tumor cell differentiation in patients with HCC undergoing liver resection when were put into the morphologic selection criteria currently in use, like the Milan criteria. (Hasegawa et al., 2008) Similar to previous research, in this study, AFP was found to make high expression of CXCR7 has more strong potential to predict the time to metastasize to lung after hepatectomy.

Because CXCR7 and CXCR4 have the common ligand, SDF-1, in this study, we also observed the prognostic roles of CXCR4 in time-to-lung metastasis. However, results indicated that CXCR4 expression was seemly a protective prognostic factor. Previous observation showed that CXCR4 was expressed lowly in HCC cell lines with metastatic potential (Mitra, De et al., 2001). Schimanski also found the decreased expression of CXCR4 in HCC cell line with strong metastatic potential such as Huh7 (Schimanski et al., 2006). On the other hand, recent research has shown that CXCR4 expression in HCC increases the risk of bone metastases and poor survival (Xiang et al., 2009). In this study, we also found the median survival time of group CXCR4^{low}CXCR7^{high} was significantly lower than group CXCR4^{low}CXCR7^{low}.

Therefore, combined these findings, we postulate that HCC cells expressed CXCR7 and CXCR4 may metastasize to lung or bone selectively.

In this study, TAT analysis showed patients with microvascular invasion expressed higher CXCR7. High CXCR7 expression in this microenviroment may promote the local vascular invasion and further metastasis. Our finding that CXCR7 has the potential role participated in the microvascular invasion is similar to the observation about breast and lung tumor. CXCR7 is highly expressed on a majority of tumor-associated blood vessels and malignant cells but not expressed on normal vasculature (Miao et al., 2007).

In conclusion, high expression of CXCR7 in tumor tissues of patients with elevated AFP level correlates with early metastasis to lung and poor potential after hepatectomy, which indicates CXCR7 is a useful prognostic factor in organ-specific metastasis of HCC. Different follow-up strategies after hepatectomy may be developed for HCC based on CXCR7 expression.

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