

Decreased expression of serum semaphorin 3B is associated with poor prognosis of patients with hepatocellular carcinoma

GUANG-ZHEN LI¹, DI SHEN², GUANG-HONG LI³, MENG WEI¹, LI-JIE ZHENG⁴, ZENG-LI LIU¹, RONG-QI SUN¹, SHAO-JUN ZHOU⁴, ZONG-LI ZHANG¹ and YAN-CHAO GAO⁵

¹Department of General Surgery, Qilu Hospital of Shandong University, Jinan, Shandong 250012;

²Department of Gynecology and Obstetrics, Shandong Maternity and Child Care Hospital, Jinan, Shandong 250014; ³Department of Endocrinology, The Affiliated Hospital of Taishan Medical College,

Tai'an, Shandong 271000; ⁴Department of General Surgery, Qilu Hospital of Shandong University,

Qingdao, Shandong 266035; ⁵Department of Hepatobiliary Surgery,

Liaocheng People's Hospital, Liaocheng, Shandong 252000, P.R. China

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Abstract. Semaphorin 3B (SEMA-3B), which belongs to the semaphorin family, has an important role in cell apoptosis and inhibition of angiogenesis. A previous study by our group revealed that SEMA-3B was downregulated in tumor tissues of patients with hepatocellular carcinoma (HCC) and exerts anti-motility and anti-invasion effects on tumor cells. However, the serum levels of SEMA-3B and their clinical significance have remained elusive; therefore, the aim of the present study was to monitor its expression in HCC and investigate its clinical significance. ELISA was used to determine the serum levels of SEMA-3B in 132 patients with HCC and 57 healthy individuals. The association between SEMA-3B and clinicopathological parameters was investigated. Serum SEMA-3B was indicated to be significantly decreased in patients with HCC as compared with that in the controls ($P < 0.05$) and it was negatively associated with tumor size ($P = 0.039$), encapsulation ($P = 0.002$) and TNM stage ($P = 0.034$). The prognosis of patients with low expression of SEMA-3B was poor. In conclusion, the results of the present study revealed that serum SEMA-3B is decreased in HCC and is negatively associated with prognosis; therefore, it may be used as a prognostic marker in HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumor types and is responsible for ~700,000 deaths annually worldwide (1). With the advances in diagnostic and therapeutic methods, the prognosis of liver cancer has improved, but the frequent recurrence and high metastasis rates of HCC adversely affect patient outcomes. Therefore, the identification of tumor markers is crucial for early diagnosis, treatment and outcome prediction in HCC.

Semaphorin 3B (SEMA-3B), which belongs to the semaphorin family, is a secreted molecule that contains a highly conserved Sema domain in the amino terminus. SEMA-3B was initially discovered as an inhibitory axonal guidance molecule, but recent studies revealed that SEMA-3B also functions as a tumor suppressor in lung, renal, gastric, breast and prostate cancers (2-7). SEMA-3B forms a complex with neuropilins (NPs) and plexins on the cell surface. Vascular endothelial growth factor (VEGF)-A also binds to NPs and this complex promotes cellular migration and promotes tumor growth (4,8-10). SEMA-3B family proteins may competitively inhibit the function of VEGF in promoting tumor angiogenesis, as they share the same transmembrane receptors for NP-1 and NP-2 (4). SEMA-3B inhibits the migration and proliferation of cancer cells; therefore, it was hypothesized that its expression levels may affect the prognosis of patients with HCC. A previous study by our group demonstrated the suppressive effect of the SEMA-3B protein on the migration and invasion ability of cancer cells *in vitro* (11).

Aberrant expression and methylation of SEMA-3B may be of value as a marker in patients with lung and renal cancer (12). The previous study by our group revealed that SEMA-3B is downregulated in tumor tissues and has a tumor suppressor role in HCC (11). The aim of the present study was to examine the clinical value of serum SEMA-3B as a tumor marker and determine whether it may be used as a prognostic marker.

Correspondence to: Professor Zong-Li Zhang, Department of General Surgery, Qilu Hospital of Shandong University, 107 Wenhua West Road, Jinan, Shandong 250012, P.R. China
E-mail: 13708933969@163.com

Professor Yan-Chao Gao, Department of Hepatobiliary Surgery, Liaocheng People's Hospital, 67 Dongchang West Road, Liaocheng, Shandong 252000, P.R. China
E-mail: lcygyyc@126.com

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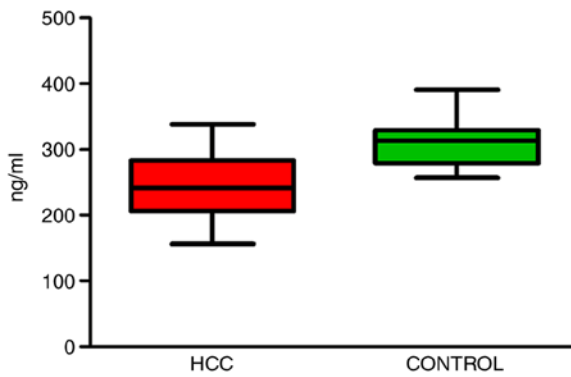


Figure 1. Serum levels of SEMA-3B are downregulated in HCC. The serum concentration of SEMA-3B was determined with ELISA in patients with HCC and healthy subjects as the control group. There was a significant difference in serum SEMA-3B between the two groups ($t=10.48$, $P<0.0001$). The serum levels of SEMA-3B were significantly downregulated in patients with HCC compared with those in the healthy controls ($242.7\pm 3.8444.06$ vs. $310.7\pm 4.3232.62$ ng/ml, respectively; $P<0.05$). SEMA-3B, semaphorin 3B; HCC, hepatocellular carcinoma.

Materials and methods

Sample collection. A total of 132 patients (73 males and 59 females, aged 37-78 years) with HCC who underwent curative surgery and were diagnosed pathologically were hospitalized at the Department of General Surgery of Qilu Hospital, Shandong University (Jinan, China) between May 2013 and December 2014, and all cases who presented during this period were enrolled. Serum samples were also collected during this period from 57 subjects who had no liver disease (healthy controls; 31 males and 26 females, aged 42-80 years). All subjects and their families agreed to participate in the present study and signed informed consent. The protocol was approved by the Ethics Committee of Qilu Hospital (Jinan, China). All patients who had a visit were contacted by telephone every month and had ultrasound or CT scan every 2-3 months. The follow-up was terminated in November 2019.

ELISA. Peripheral blood (5 ml) was collected from each of the subjects, centrifuged at $3000 \times g$ for 15 min at 4°C and the serum was separated and stored in the refrigerator at -80°C until use. The serum SEMA-3B was examined by ELISA (DLdevelop; cat. no. DL-SEMA3B-Hu) according to the manufacturer's protocol.

Statistical analysis. For statistical comparison of count data between SEMA-3B expression and clinical indicators, the χ^2 test was used. The levels of SEMA-3B between patients with HCC and controls were compared using Student's t-test. Survival curves were constructed using the Kaplan-Meier survival analysis method and the differences in survival curves were compared with the log-rank test. All the statistical analyses were performed with SPSS 18.0 statistical software (SPSS Inc.) and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Serum SEMA-3B is downregulated in HCC. The expression of SEMA-3B in the serum of patients with HCC and healthy

Table I. Clinicopathological variables associated with different expression patterns of serum SEMA-3B.

| Variable | Number of patients | | P-value |
|-------------------------|--------------------|-----|---------|
| | High | Low | |
| Sex | | | 0.382 |
| Male | 33 | 40 | |
| Female | 32 | 27 | |
| Age (years) | | | 0.229 |
| ≤ 60 | 28 | 36 | |
| > 60 | 37 | 31 | |
| HBsAg status | | | 0.374 |
| Negative | 22 | 28 | |
| Positive | 43 | 39 | |
| AFP (ng/ml) | | | 0.163 |
| ≤ 20 | 30 | 25 | |
| > 20 | 35 | 42 | |
| Liver cirrhosis | | | 0.209 |
| Negative | 20 | 28 | |
| Positive | 45 | 39 | |
| Cell differentiation | | | 0.475 |
| Moderate/poor | 43 | 40 | |
| High | 22 | 27 | |
| Number of tumors | | | 0.008 |
| Solitary | 41 | 25 | |
| Multiple | 24 | 40 | |
| Largest tumor size (cm) | | | 0.039 |
| ≤ 5 | 39 | 28 | |
| > 5 | 26 | 40 | |
| Tumor capsule | | | 0.002 |
| Absent | 40 | 22 | |
| Present | 25 | 45 | |
| CLIP score (points) | | | 0.025 |
| 0-1 | 41 | 29 | |
| ≥ 2 | 24 | 38 | |
| TNM stage | | | 0.034 |
| I | 36 | 23 | |
| II/III | 29 | 42 | |

SEMA-3B, semaphorin 3B; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; AFP, α -fetoprotein; CLIP, Cancer of the Liver Italian Program.

controls was measured to determine whether there was any difference between the two groups. ELISA was used to monitor the expression of serum SEMA-3B. The serum levels of SEMA-3B were indicated to be significantly downregulated in patients with HCC compared with those in the controls (242.7 ± 44.06 vs. 310.7 ± 32.62 ng/ml, respectively; $P<0.05$; Fig. 1).

Association of clinicopathological variables with different expression patterns of serum SEMA-3B. HCC cases were

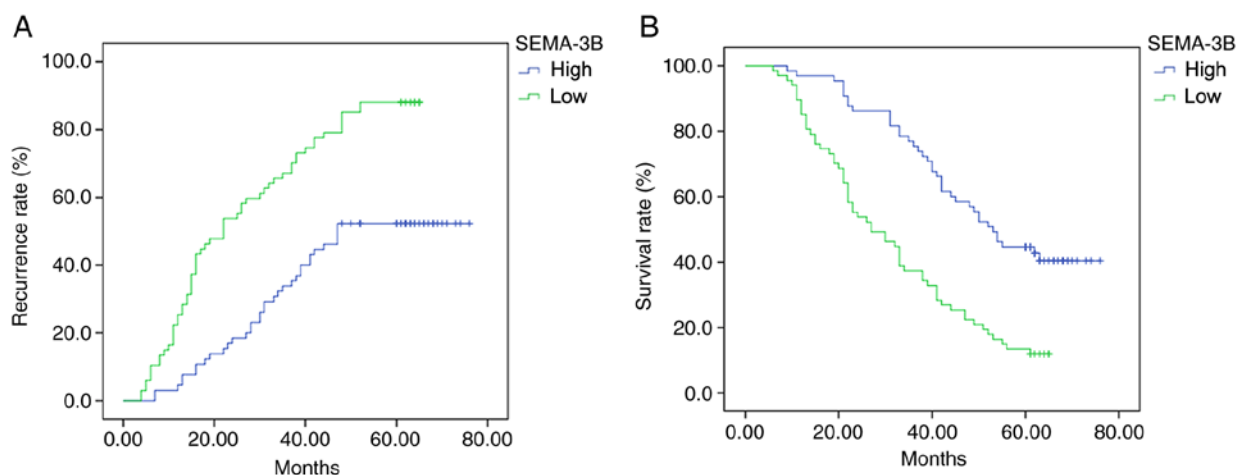


Figure 2. Prognostic significance of SEMA-3B in HCC. (A) The recurrence rate in the low group was significantly lower than that in the high group ($P < 0.001$) and (B) the survival rate in the high group was significantly higher than that in the low group ($P < 0.001$). For patients who were alive and did not relapse at the last follow-up, they are treated as censored data during the statistical analysis. SEMA-3B, semaphorin 3B; HCC, hepatocellular carcinoma.

divided into two groups according to the mean expression of serum SEMA-3B (cut-off, 242.7 ng/ml). The expression of serum SEMA-3B was high in 65 and low in 67 HCC patients. Serum SEMA-3B was negatively associated with the number of tumors, largest tumor size, presence of tumor capsule, Cancer of the Liver Italian Program (CLIP) score (13) and the TNM stage ($P < 0.05$; Table I).

Serum SEMA-3B may be used as a prognostic marker for HCC. Clinical follow-up of the patients revealed that the cancer recurrence rate in the low-expression group was significantly lower compared with that in the high-expression group ($P < 0.001$; Fig. 2A). The recurrence rates of the high-expression group at 1, 3 and 5 years were 4.62, 29.23 and 60.00%, respectively. The recurrence rates of the low-expression groups were 25.37, 67.16 and 88.06%, respectively. Furthermore, the survival rate in the high-expression group was significantly higher compared with that in the low-expression group ($P < 0.001$; Fig. 2B): 1-year survival rate 96.92 vs. 83.58%, respectively; 3-year survival rate 72.308 vs. 53.73%, respectively; and 5-year survival rate 38.46 vs. 31.34%, respectively.

Discussion

As HCC is characterized by low sensitivity to radiotherapy and chemotherapy, the prognosis of the patients with HCC is poor (14). Despite advances in the development of early detection methodologies, the questionable effectiveness and high cost of the procedures available for the treatment of HCC pose a challenge for disease management. Therefore, it is crucial to identify novel therapeutic targets and diagnostic biomarkers to ensure timely treatment and improve survival rates. In the present study, the clinical value of serum SEMA-3B was assessed and it was discussed whether it may be of value in the treatment of HCC.

SEMA-3B, encoded at chromosome 3p21.3, is a secreted molecule that contains a highly conserved Sema domain in the amino terminus. SEMA-3B was initially discovered as an inhibitory axonal guidance molecule and it was also recently recognized as a candidate tumor suppressor gene (15-17). Loss

of SEMA-3B expression in cells of the tumor microenvironment, as well as tumor cells themselves, may increase the aggressiveness of breast cancer (7). In a previous study by our group, the expression of SEMA-3B protein was investigated in HCC and normal tissues and the expression of SEMA-3B was indicated to be significantly downregulated in HCC tissues (11). To the best of our knowledge, no previous studies have reported the serum levels of SEMA-3B and SEMA-3F in patients with HCC to date; therefore, ELISA was used to monitor the expression of serum SEMA-3B and SEMA-3F, but only the expression of serum SEMA-3B was observed to be decreased compared with that in healthy controls. Several mechanisms are involved in decreasing the expression of SEMA-3B. Methylation participates in the downregulation of SEMA-3B (18). Introduction of exogenous p53 into a glioblastoma cell line lacking wild-type p53, U373MG, markedly induced the expression of SEMA-3B mRNA, as SEMA-3B is a direct target of p53 (19).

Based on the expression of serum SEMA-3B in patients with HCC, the patients were followed-up. A χ^2 test for the association of clinicopathological parameters with the serum levels of SEMA-3B (high vs. low) revealed that the expression levels of SEMA-3B were significantly associated with the formation of a capsule, possibly via the inhibitory effect of SEMA-3B on the formation of vessels (20). SEMA-3B is able to inhibit the expression and activity of MMP-2 and MMP-9 (21,22). SEMA-3B family proteins may competitively inhibit the function of VEGF in promoting tumor angiogenesis, as they share the same transmembrane receptors of NP-1 and NP-2 (4). The expression of NP-1 was reported to be associated with the clinicopathologic parameters of patients with cholangiocarcinoma (23). The positive rates of NP-1 in HCC tissues and adjacent tissues were 63.0 and 4.1%, and a study by our group also indicated that the expression of NP-1 in HCC is associated with clinicopathologic parameters (24). The previous study (11) by our group demonstrated a negative association between the level of SEMA-3B protein expression and microvascular density, which may reflect the fact that SEMA-3B has an important role in angiogenesis. In addition, serum SEMA-3B was indicated to be negatively correlated with the number of tumors, largest tumor size, tumor

capsule, CLIP score and TNM stage in the present study. As the number of tumors, largest tumor size, tumor capsule, CLIP score and TNM stage are closely associated with the prognosis of the patients (25), it was inferred that serum SEMA-3B is a tumor suppressor gene.

In order to determine the possible influence of the serum levels of SEMA-3B on prognosis, patients were divided into two groups, namely a high- and a low-expression group. Analysis of the follow-up data of the patients of the present study revealed that the recurrence rate in the high-expression group was markedly lower compared with that in the low-expression group, while the cumulative survival rate was significantly higher compared with that of the low-expression group. Suppression of miR-221 was previously reported to inhibit glioma cells by targeting SEMA-3B, and SEMA-3B inhibited cell proliferation and invasion (26). SEMA-3B was also indicated to be associated with a favorable survival prognosis for patients with esophageal squamous cell carcinoma due to upregulating p53 and p21 (27). A previous study by our group confirmed that SEMA-3B inhibits angiogenesis and cell migration, and SEMA-3B levels in HCC tissue may be used as a marker to predict prognosis (11). Therefore, serum SEMA-3B may be a valuable indicator for predicting the prognosis of patients with HCC.

In conclusion, serum SEMA-3B may be easily detected in the peripheral blood and may be used for the diagnosis and prediction of prognosis of patients with HCC. The exact role of SEMA-3B in cancer development and underlying mechanisms of action require further elucidation, but the results of the present study indicated the potentially important role of serum SEMA-3B in the diagnosis and prediction of prognosis of patients with HCC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZLZ and YCG conceived the study and drafted the manuscript. GZL enrolled subjects and compared their data. DS, GHL, MW, LJZ, ZLL, RQS and SJZ performed ELISA and collected data. All authors read and approved the final manuscript. ZLZ and YCG confirmed the authenticity of the raw data.

Ethics approval and consent to participate

This study was approved by the ethics committee of Qilu Hospital (Jinan, China). Patients who participated in this study, provided written informed consent and had complete clinical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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