Acta Medica Okayama

Volume 63, *Issue* 6 2009 *Article* 1

DECEMBER 2009

Significance of Des-gamma-carboxy Prothrombin Production in Hepatocellular Carcinoma

Tatsuya Fujikawa* Hidenori Shiraha†

Kazuhide Yamamoto‡

*Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,

†Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, hshiraha@md.okayama-u.ac.jp

‡Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,

Significance of Des-gamma-carboxy Prothrombin Production in Hepatocellular Carcinoma

Tatsuya Fujikawa, Hidenori Shiraha, and Kazuhide Yamamoto

Abstract

Serum des-gamma-carboxy prothrombin (DCP) is commonly used to detect hepatocellular carcinoma (HCC). This review focuses on the clinical features of DCP-positive HCC and the molecular function of DCP in HCC. DCP-positive HCC demonstrates more aggressive clinicopathological features than DCP-negative HCC. Analysis of the biological effects of DCP revealed that DCP acts as a growth factor in both an autocrine and paracrine manner. DCP stimulates HCC cell proliferation through the Met-Janus kinase 1-signal transducer and activator of transcription 3 signaling pathway, whereas for vascular endothelial cells, it stimulates cell proliferation and migration through the kinase insert domain receptor-phospholipase C-gamma-mitogen-activated protein kinase signaling pathway.

KEYWORDS: des-gamma-carboxy prothrombin, hepatocellular carcinoma, signaling pathway, cell proliferation, angiogenesis

Acta Med. Okayama, 2009
Vol. 63, No. 6, pp. 299-304

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Review

Significance of Des-gamma-carboxy Prothrombin Production in Hepatocellular Carcinoma

Tatsuya Fujikawa[§], Hidenori Shiraha*, and Kazuhide Yamamoto

Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

Serum des-γ-carboxy prothrombin (DCP) is commonly used to detect hepatocellular carcinoma (HCC). This review focuses on the clinical features of DCP-positive HCC and the molecular function of DCP in HCC. DCP-positive HCC demonstrates more aggressive clinicopathological features than DCP-negative HCC. Analysis of the biological effects of DCP revealed that DCP acts as a growth factor in both an autocrine and paracrine manner. DCP stimulates HCC cell proliferation through the Met-Janus kinase 1-signal transducer and activator of transcription 3 signaling pathway, whereas for vascular endothelial cells, it stimulates cell proliferation and migration through the kinase insert domain receptor-phospholipase C-γ-mitogen-activated protein kinase signaling pathway.

Key words: des-γ-carboxy prothrombin, hepatocellular carcinoma, signaling pathway, cell proliferation, angiogenesis

Hepatocellular carcinoma (HCC) is one of the most common abdominal cancers in the world [1, 2]. The number of deaths per year due to HCC is estimated to be over 30,000 in Japan [3] and over one half million worldwide [4]. Recently, early-stage HCC has been treated by surgical resection or radio-frequency ablation, and the prognosis for patients receiving these therapies has improved [5]. However, there is no effective therapy for patients with advanced HCC [5]; therefore, early detection of HCC is important.

Tumor markers are widely used for early detection of HCC. Alpha-fetoprotein (AFP), a primary tumor marker for HCC, has been used to detect and monitor HCC [6-9]. In general, the sensitivity of AFP is acceptable, though it is not always acceptable for patients with early HCC, since the AFP levels are sometimes low in these patients [10, 11]. Des-γ-carboxy prothrombin (DCP) is also commonly used to detect HCC. DCP is an abnormal prothrombin without carboxylation of 10 glutamic acid residues at its N-terminus and is devoid of coagulation activity [12]. Due to the lack of carboxylation of the carbon atom at the γ-position, it is called des-γ-carboxy prothrombin. In 1984, Liebman *et al.* [13] first reported that serum DCP levels are increased in patients with HCC based on a competitive radioimmunoassay.

Because AFP and DCP behave independently in serum, they can complement each other for the diagnosis of HCC [9, 14-16]. Hence, the combined use of AFP and DCP increases sensitivity as well as specificity and has been commonly used in the diagnosis of HCC [17, 18]. Although AFP is superior to DCP as a diagnostic tool for early detection of HCC, DCP is more reliable than AFP as a prognostic tool

Received May 7, 2009; accepted July 30, 2009.
*Corresponding author. Phone:+81-86-235-7219; Fax:+81-86-225-5991
E-mail:hshiraha@md.okayama-u.ac.jp (H. Shiraha)
[§]The winner of the 2007 Yamada Prize of the Okayama Medical Association.

for HCC in terms of reflecting the invasive characteristics [19, 20].

This review focuses on the association between DCP and the clinical development of HCC. We also describe our series of reports concerning the effect of DCP on the development of HCC.

Serum DCP is a Prognostic Factor for HCC

DCP has been reported to be a clinical prognostic factor for recurrence and survival after hepatectomy [15, 21–24], liver transplantation [25, 26], radiofrequency ablation treatment [27–29], and transarterial chemoembolization treatment [30]. These findings are based on the fact that the serum DCP level correlates with tumor diameter, which reflects HCC cell growth [31, 32].

Serum DCP Level and Portal Vein Invasion by HCC

The serum DCP level correlates with the number of tumors, the histological tumor grade of HCC differentiation, the development of portal vein invasion, and tumor size [23, 32–34]. The serum DCP level is the most significant predictor for the development of portal vein invasion [35–37]. Consistent with these reports, there is a positive correlation between serum DCP level and vascular invasion in adult HCC patients who undergo living donor liver transplantation [25]. In this report, it was demonstrated that the incidence of positive histological vascular invasion in patients with a DCP level above 300 mAU (arbitrary units)/mL was significantly higher than that in those with a DCP level below 300 mAU/mL, demonstrating that high serum DCP correlates with histological vascular invasion and could be a strong prognostic indicator.

Characteristics of DCP-predominant HCC

A milder form of hepatitis is the source of HCC in patients with HCV-related HCC, and DCP-predominant HCC is associated with a more stable platelet count than AFP-predominant HCC [38]. Among patients with HBV-related HCC, those with a high serum DCP level have more aggressive tumor characteristics and a shorter median survival time than those with a low serum DCP level [39]. Even after excluding patients with Child-Pugh class C and advanced tumor stages at diagnosis, a high DCP level is still an independent predictor of survival, suggesting that the hepatocarcinogenic mechanisms in DCP-predominant HCC may differ from those in AFP-predominant HCC.

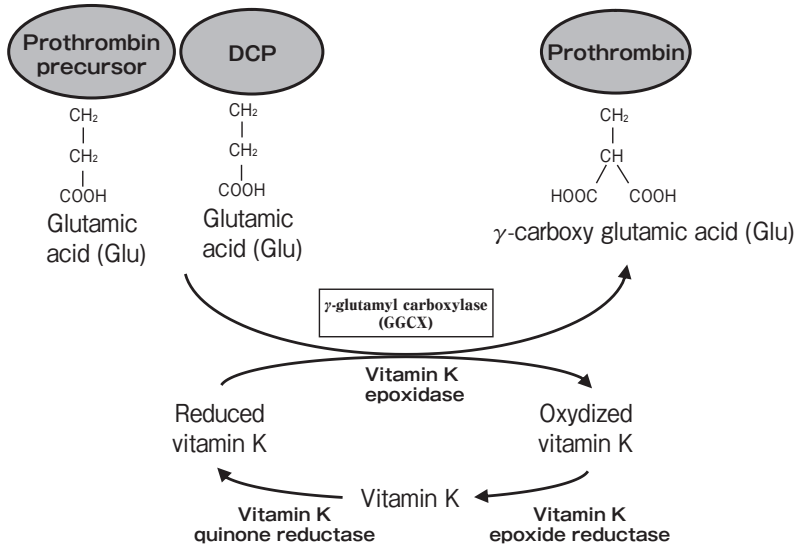

Fig. 1 The vitamin K-dependent carboxylation reaction. The glutamic acid residues (Glu) of the prothrombin precursor and des-γ-carboxy prothrombin (DCP) are carboxylated into gamma-carboxy glutamic acid (Gla) residues by GGCX, which requires reduced vitamin K as a cofactor. Normal prothrombin has 10 Gla residues, and all are required for the coagulation activity.

Molecular Basis for DCP Production in HCC

Vitamin K-dependent γ-glutamyl carboxylase (GGCX) plays a crucial role in converting DCP into normal prothrombin via vitamin K epoxide reductase (VKOR) [12, 40, 41]. GGCX mediates the post-translational modification required for the activity of all vitamin K-dependent proteins including prothrombin [42–44] (Fig. 1). It has been reported that DCP-producing HCC shows low GGCX activity [45], and a point mutation of the GGCX gene causes insufficient GGCX enzyme activity in patients with blood coagulation disorders [46–49].

However, there are no reports referring to allelic variants of GGCX in HCC. We performed a cDNA sequence analysis of GGCX using 2 groups of HCC cell lines classified as DCP-positive (Hep3B, HepG2, HuH1, HuH7 and PLC) and DCP-negative (SK-Hep-1, HLE, HLF and JHH1), but neither a point mutation nor a missense mutation was found [50], and a sequence analysis with a sense primer did not detect a sequence in DCP-positive HCC cell lines. In contrast, a sequence analysis with an antisense primer in DCP-positive HCC cell lines revealed that 2 waveforms of the sequence overlapped from positions 162 to 213. These overlapped sequences were identified as the exon 1 and exon 2 sequences of GGCX by a BLAST (NCBI) homology search analysis. The minor sequence was identified as a GGCX alternative splicing variant lacking exon 2 (Δ2GGCX) (Fig. 2). When the HCC cell lines were analyzed, only Δ2GGCX was expressed in the DCP-positive HCC cell lines.

We further examined the function of Δ2GGCX and demonstrated that transfection of a low proportion of Δ2GGCX cDNA causes DCP production in DCP-negative HCC cell lines [50], suggesting that Δ2GGCX could function as a dominant-negative inhibitor of wild type GGCX. It may be that the GGCX structure is altered in Δ2GGCX, which affects the regions responsible for the active site or the binding site of coenzymes, including VKOR and calumenin [51–54].

Another possible mechanism for DCP production has been reported in some studies. The uptake of vitamin K, a GGCX coenzyme, is suppressed in 2 rat hepatoma cell lines, MH7777 and H4IIE [51]. DCP production due to a deficiency of vitamin K has also been reported in HCC [45, 55, 56].

DCP Stimulates Cell Proliferation in HCC Cell Lines

Many studies have reported that serum and tissue DCP expression reflect the biological malignant potential of HCC [21, 22, 33, 57]. However, there are no reports concerning the direct effect of DCP on HCC cell proliferation. We demonstrated that purified DCP stimulates DNA synthesis in HCC cell lines (Hep3B and SK-Hep-1) in a dose-dependent manner [58]. While exploring the signaling pathway for DCP stimulation, we identified Met, a hepatocyte growth factor receptor, as a receptor for DCP in HCC cell lines [58]. The transductional apparatus of DCP via Met was determined to activate the Janus kinase 1 (JAK1)-signal transducer and activator of transcription 3 (STAT3) signaling pathway during cell proliferation (Fig. 3), indicating that a novel autocrine/paracrine growth stimulatory mechanism is associated with the development of HCC.

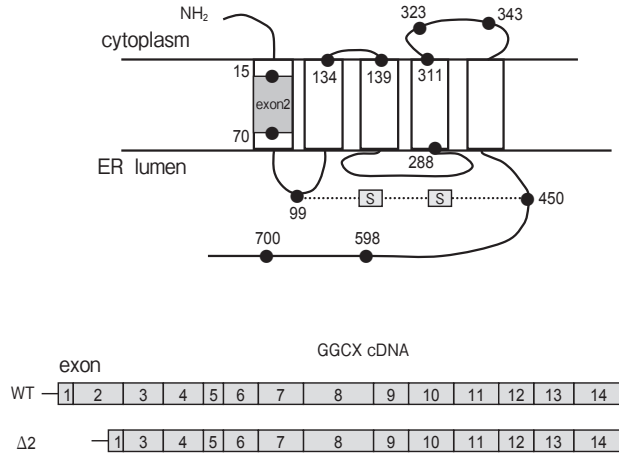

Fig. 2 A splicing variant of vitamin K-dependent γ-glutamyl carboxylase was identified in hepatocellular carcinoma cell lines. Top panel, structure of the vitamin K-dependent γ-glutamyl carboxylase (GGCX) transmembrane protein. Exon 2 is located in the first GGCX transmembrane domain. Bottom panel, the gene structure of GGCX derived from hepatocellular carcinoma (HCC) cell lines. The GGCX variant was expressed in des-γ-carboxy prothrombin-positive HCC and has an exon 2 deletion.

DCP Promotes Vascular Endothelial Cell Proliferation and Migration

Because HCC tumor arterial vascularity strongly correlates with the expression of serum and tissue DCP [59], DCP secreted from HCC cells may act as a paracrine interaction factor between HCC cells and vascular endothelial cells.

Hence, we assessed the effect of DCP on human umbilical vein endothelial cell (HUVEC) proliferation and migration as a representative part of angiogenesis and found that DCP stimulates HUVEC proliferative activity [60].

Unlike in HCC cells, DCP stimulates cell migratory activity in HUVEC; however, there was no Met protein expression or JAK1-STAT3 signaling activation in DCP-treated HUVEC. An antibody array analysis revealed that the kinase insert domain receptor (KDR), also known as vascular endothelial growth factor receptor 2, acts as a DCP receptor in HUVEC.

The DCP transductional apparatus was shown to activate the phospholipase C-γ (PLC-γ)-mitogen-activated protein kinase (MAPK) signaling pathway via activation of KDR (Fig. 3). Together, these findings demonstrate a novel paracrine mechanism that is involved in the development of HCC-associated angiogenesis.

Conclusions

DCP is a useful prognostic tumor marker for HCC, because DCP-positive HCC demonstrates more aggressive clinicopathological features than DCP-negative HCC. The dominant-negative effect of Δ2GGCX may be the molecular mechanism for DCP production. DCP secretion both acts as an autocrine driver of HCC cells and exerts a paracrine influence on vascular endothelial cell proliferation and migration with respect to angiogenesis.

Acknowledgments. We acknowledge Drs. Yasushi Shiratori, Naoki Ueda, and Mayumi Suzuki for their assistance and useful discussions.

References

1. El-Serag HB and Rudolph KL: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology (2007) 132: 2557–2576.
2. Addeo R, Caraglia M and Del Prete S: Highlights of regional meeting of Italian Southern Oncological Group (GOIM): focus on hepatocellular carcinoma: biological and clinical background, therapeutic guide-lines and perspectives. 7 November 2008, Naples, Italy. Expert Opin Investig Drugs (2009) 18: 373–378.
3. Omata M: Modalities of prevention of recurrence after tumor ablation/resection of HCC. J Gastroenterol Hepatol (2004) 19: S270.
4. Hussain K and El-Serag HB: Epidemiology, screening, diagnosis and treatment of hepatocellular carcinoma. Minerva Gastroenterol Dietol (2009) 55: 123–138.

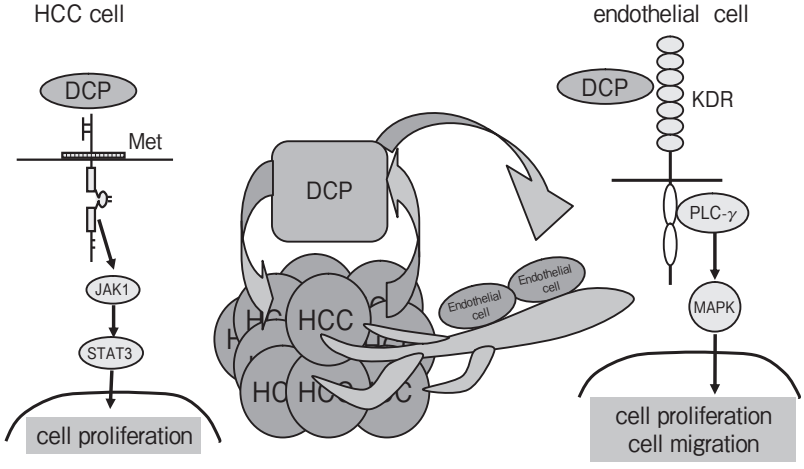

Fig. 3 Des-γ-carboxy prothrombin has the potential for both autocrine and paracrine stimulation in the development of hepatocellular carcinoma. Secreted des-γ-carboxy prothrombin (DCP) binds to Met on hepatocellular carcinoma (HCC) cells and stimulates the Met-Janus kinase 1-signal transducer and activator of transcription 3 (Met-JAK1-STAT3) signaling pathway, which results in HCC cell proliferation. In contrast, secreted DCP binds to kinase insert domain receptor (KDR) on endothelial cells and stimulates the KDR-phospholipase C-γ-mitogen-activated protein kinase (KDR-PLC-γ-MAPK) signaling pathway. This stimulation results in endothelial cell proliferation and cell migration.

5. Nakakura EK and Choti MA: Management of hepatocellular carcinoma. Oncology (Williston Park) (2000) 14: 1085–1098; discussion 1098–1102.
6. Izumi R, Shimizu K, Kiriyama M, Hashimoto T, Urade M, Yagi M, Mizukami Y, Nonomura A and Miyazaki I: Alpha-fetoprotein production by hepatocellular carcinoma is prognostic of poor patient survival. J Surg Oncol (1992) 49: 151–155.
7. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y and Nagataki S: Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med (1993) 328: 1802–1806.
8. Ramsey WH and Wu GY: Hepatocellular carcinoma: update on diagnosis and treatment. Dig Dis (1995) 13: 81–91.
9. Ishii M, Gama H, Chida N, Ueno Y, Shinzawa H, Takagi T, Toyota T, Takahashi T and Kasukawa R: Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. Am J Gastroenterol (2000) 95: 1036–1040.
10. Sherman M: Alphafetoprotein:an obituary. J Hepatol (2001) 34: 603–605.
11. Ward J and Robinson PJ: How to detect hepatocellular carcinoma in cirrhosis. Eur Radiol (2002) 12: 2258–2272.
12. Uehara S, Gotoh K, Handa H, Honjo K and Hirayama A: Process of carboxylation of glutamic acid residues in the gla domain of human des-gamma-carboxyprothrombin. Clin Chim Acta (1999) 289: 33–44.
13. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS and Furie B: Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med (1984) 310: 1427–1431.
14. Fujiyama S, Morishita T, Hashiguchi O and Sato T: Plasma abnormal prothrombin (des-gamma-carboxy prothrombin) as a marker of hepatocellular carcinoma. Cancer (1988) 61: 1621–1628.
15. Nakao A, Taniguchi K, Inoue S, Harada A, Nonami T, Watanabe K and Takagi H: Usefulness of simultaneous determination of alpha-fetoprotein and des-gamma-carboxy prothrombin in hepatocellular carcinoma. Semin Surg Oncol (1996) 12: 160–163.
16. Giannelli G and Antonaci S: New frontiers in biomarkers for hepatocellular carcinoma. Dig Liver Dis (2006) 38: 854–859.
17. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Takasaki K, Takenami K, Yamamoto M and Nakano M: Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. Cancer (2000) 88: 544–549.
18. Soga K, Watanabe T, Aikawa K, Toshima M, Shibasaki K and Aoyagi Y: Serum des-gamma-carboxyprothrombin level by a modified enzyme immunoassay method in hepatocellular carcinoma: clinical significance in small hepatocellular carcinoma. Hepatogastroenterology (1998) 45: 1737–1741.
19. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Yamamoto M, Takasaki K and Nakano M: Comparison of clinicopathological features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein alone and those seropositive for des-gamma-carboxy prothrombin alone. J Gastroenterol Hepatol (2001) 16: 1290–1296.
20. Hamamura K, Shiratori Y, Shiina S, Imamura M, Obi S, Sato S, Yoshida H and Omata M: Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. Cancer (2000) 88: 1557–1564.
21. Inoue S, Nakao A, Harada A, Nonami T and Takagi H: Clinical significance of abnormal prothrombin (DCP) in relation to postoperative survival and prognosis in patients with hepatocellular carcinoma. Am J Gastroenterol (1994) 89: 2222–2226.
22. Suehiro T, Matsumata T, Itasaka H, Taketomi A, Yamamoto K and Sugimachi K: Des-gamma-carboxy prothrombin and proliferative activity of hepatocellular carcinoma. Surgery (1995) 117: 682–691.
23. Imamura H, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, Miyagawa S, Makuuchi M and Kawasaki S: Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. Br J Surg (1999) 86: 1032–1038.
24. Nanashima A, Morino S, Yamaguchi H, Tanaka K, Shibasaki S, Tsuji T, Hidaka S, Sawai T, Yasutake T and Nakagoe T: Modified CLIP using PIVKA-II for evaluating prognosis after hepatectomy for hepatocellular carcinoma. Eur J Surg Oncol (2003) 29: 735–742.
25. Shimada M, Yonemura Y, Ijichi H, Harada N, Shiotani S, Ninomiya M, Terashi T, Yoshizumi T, Soejima Y and Maehara Y: Living donor liver transplantation for hepatocellular carcinoma: a special reference to a preoperative des-gamma-carboxy prothrombin value. Transplant Proc (2005) 37: 1177–1179.
26. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uhciyama H, Ikegami T, Harada N, Yamashita Y, Sugimachi K, Kayashima H, Iguchi T and Maehara Y: Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. Transplantation (2009) 87: 531–537.
27. Takahashi S, Kudo M, Chung H, Inoue T, Ishikawa E, Kitai S, Tatsumi C, Ueda T, Nagai T, Minami Y and Ueshima K: PIVKA-II is the best prognostic predictor in patients with hepatocellular carcinoma after radiofrequency ablation therapy. Oncology (2008) 75 Suppl 1: 91–98.
28. Toyoda H, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, Oka H, Yamazaki O, Manabe T, Urano F, Chung H, Kudo M and Matsunaga T: Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. J Hepatol (2008) 49: 223–232.
29. Kobayashi M, Ikeda K, Kawamura Y, Yatsuji H, Hosaka T, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y and Kumada H: High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. Cancer (2009) 115: 571–580.
30. Maeda S, Fujiyama S, Tanaka M, Ashihara H, Hirata R and Tomita K: Survival and local recurrence rates of hepatocellular carcinoma patients treated by transarterial chemolipiodolization with and without embolization. Hepatol Res (2002) 23: 202–210.
31. Nakao A, Suzuki Y, Isshiki K, Kimura Y, Takeda S, Kishimoto W, Nonami T, Harada A and Takagi H: Clinical evaluation of plasma abnormal prothrombin (des-gamma-carboxy prothrombin) in hepatobiliary malignancies and other diseases. Am J Gastroenterol (1991) 86: 62–66.
32. Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R and Tomita K: Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. Oncology (2002) 62 Suppl 1: 57–63.
33. Shimada M, Takenaka K, Fujiwara Y, Gion T, Kajiyama K, Maeda T, Shirabe K and Sugimachi K: Des-gamma-carboxy prothrombin and alpha-fetoprotein positive status as a new prognostic indicator after hepatic resection for hepatocellular carcinoma.

Cancer (1996) 78: 2094–2100.
34. Miyaaki H, Nakashima O, Kurogi M, Eguchi K and Kojiro M: Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. J Gastroenterol (2007) 42: 962–968.
35. Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, Yoshida H, Shiina S and Omata M: Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. Cancer (2001) 91: 561–569.
36. Hagiwara S, Kudo M, Kawasaki T, Nagashima M, Minami Y, Chung H, Fukunaga T, Kitano M and Nakatani T: Prognostic factors for portal venous invasion in patients with hepatocellular carcinoma. J Gastroenterol (2006) 41: 1214–1219.
37. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Yamaguchi A, Isogai M, Kaneoka Y and Washizu J: Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol (2006) 4: 111–117.
38. Yano Y, Yamashita F, Kuwaki K, Fukumori K, Kato O, Yamamoto H, Ando E, Tanaka M and Sata M: Clinical features of hepatitis C virus-related hepatocellular carcinoma and their association with alpha-fetoprotein and protein induced by vitamin K absence or antagonist-II. Liver Int (2006) 26: 789–795.
39. Kim HS, Park JW, Jang JS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY and Jang MK: Prognostic Values of alpha-fetoprotein and Protein Induced by Vitamin K Absence or Antagonist-II in Hepatitis B Virus-related Hepatocellular Carcinoma: A Prospective Study. J Clin Gastroenterol (2009) 43: 482–488.
40. Wu SM, Cheung WF, Frazier D and Stafford DW: Cloning and expression of the cDNA for human gamma-glutamyl carboxylase. Science (1991) 254: 1634–1636.
41. Wu SM, Morris DP and Stafford DW: Identification and purification to near homogeneity of the vitamin K-dependent carboxylase. Proc Natl Acad Sci USA (1991) 88: 2236–2240.
42. Liu Y, Nelson AN and Lipsky JJ: Vitamin K-dependent carboxylase: mRNA distribution and effects of vitamin K-deficiency and warfarin treatment. Biochem Biophys Res Commun (1996) 224: 549–554.
43. Romero EE, Deo R, Velazquez-Estades LJ and Roth DA: Cloning, structural organization, and transcriptional activity of the rat vitamin K-dependent gamma-glutamyl carboxylase gene. Biochem Biophys Res Commun (1998) 248: 783–788.
44. Wu SM, Stafford DW, Frazier LD, Fu YY, High KA, Chu K, Sanchez-Vega B and Solera J: Genomic sequence and transcription start site for the human gamma-glutamyl carboxylase. Blood (1997) 89: 4058–4062.
45. Huisse MG, Leclercq M, Belghiti J, Flejou JF, Suttie JW, Bezeaud A, Stafford DW and Guillin MC: Mechanism of the abnormal vitamin K-dependent gamma-carboxylation process in human hepatocellular carcinomas. Cancer (1994) 74: 1533–1541.
46. Brenner B, Sanchez-Vega B, Wu SM, Lanir N, Stafford DW and Solera J: A missense mutation in gamma-glutamyl carboxylase gene causes combined deficiency of all vitamin K-dependent blood coagulation factors. Blood (1998) 92: 4554–4559.
47. Rost S, Fregin A, Koch D, Compes M, Muller CR and Oldenburg J: Compound heterozygous mutations in the gamma-glutamyl carboxylase gene cause combined deficiency of all vitamin K-dependent blood coagulation factors. Br J Haematol (2004) 126: 546–549.
48. Spronk HM, Farah RA, Buchanan GR, Vermeer C and Soute BA: Novel mutation in the gamma-glutamyl carboxylase gene resulting in congenital combined deficiency of all vitamin K-dependent blood coagulation factors. Blood (2000) 96: 3650–3652.
49. Tagawa M, Omata M and Ohto M: Nucleotide sequence of prothrombin gene in abnormal prothrombin-producing hepatocellular carcinoma cell lines. Cancer (1992) 69: 643–647.
50. Ueda N, Shiraha H, Fujikawa T, Takaoka N, Nakanishi Y, Suzuki M, Matsuo N, Tanaka S, Nishina S, Uemura M, Takaki A, Shiratori Y and Yamamoto K: Exon 2 deletion splice variant of g-glutamyl carboxylase causes des-g-carboxy prothrombin production in hepatocellular carcinoma cell lines. Mol Oncol (2008) 2: 241–249.
51. Li ZQ, He FY, Stehle CJ, Wang Z, Kar S, Finn FM and Carr BI: Vitamin K uptake in hepatocytes and hepatoma cells. Life Sci (2002) 70: 2085–2100.
52. Otsuka M, Kato N, Shao RX, Hoshida Y, Ijichi H, Koike Y, Taniguchi H, Moriyama M, Shiratori Y, Kawabe T and Omata M: Vitamin K2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. Hepatology (2004) 40: 243–251.
53. Price PA and Williamson MK: Substrate recognition by the vitamin K-dependent gamma-glutamyl carboxylase: identification of a sequence homology between the carboxylase and the carboxylase recognition site in the substrate. Protein Sci (1993) 2: 1987–1988.
54. Pudota BN, Hommema EL, Hallgren KW, McNally BA, Lee S and Berkner KL: Identification of sequences within the gamma-carboxylase that represent a novel contact site with vitamin K-dependent proteins and that are required for activity. J Biol Chem (2001) 276: 46878–46886.
55. Furukawa M, Nakanishi T, Okuda H, Ishida S and Obata H: Changes of plasma des-gamma-carboxy prothrombin levels in patients with hepatocellular carcinoma in response to vitamin K. Cancer (1992) 69: 31–38.
56. Okuda H, Obata H, Nakanishi T, Furukawa R and Hashimoto E: Production of abnormal prothrombin (des-gamma-carboxy prothrombin) by hepatocellular carcinoma. A clinical and experimental study. J Hepatol (1987) 4: 357–363.
57. Sakon M, Monden M, Gotoh M, Kanai T, Umeshita K, Nakano Y, Mori T, Sakurai M and Wakasa K: Relationship between pathologic prognostic factors and abnormal levels of des-gamma-carboxy prothrombin and alpha-fetoprotein in hepatocellular carcinoma. Am J Surg (1992) 163: 251–256.
58. Suzuki M, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, Koike K, Takaki A and Shiratori Y: Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. J Biol Chem (2005) 280: 6409–6415.
59. Tamano M, Sugaya H, Oguma M, Iijima M, Yoneda M, Murohisa T, Kojima K, Kuniyoshi T, Majima Y, Hashimoto T and Terano A: Serum and tissue PIVKA-II expression reflect the biological malignant potential of small hepatocellular carcinoma. Hepatol Res (2002) 22: 261–269.
60. Fujikawa T, Shiraha H, Ueda N, Takaoka N, Nakanishi Y, Matsuo N, Tanaka S, Nishina S, Suzuki M, Takaki A, Sakaguchi K and Shiratori Y: Des-gamma-carboxyl prothrombin-promoted vascular endothelial cell proliferation and migration. J Biol Chem (2007) 282: 8741–8748.