

Review

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

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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

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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], radio-frequency 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 300mAU (arbitrary units)/mL was significantly higher than that in those with a DCP level below 300mAU/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.

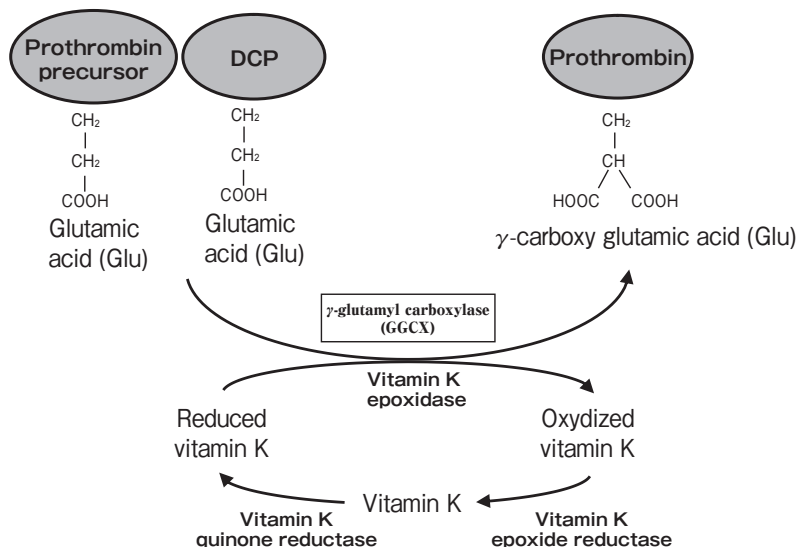


Fig. 1 The vitamin K-dependent carboxylation reaction. The glutamic acid residues (Glu) of the prothrombin precursor and des- γ -carboxy prothrombin (DCP) are carboxylated into gamma-carboxy glutamic acid (Gla) residues by GGCX, which requires reduced vitamin K as a cofactor. Normal prothrombin has 10 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 ($\Delta 2$ GGCX) (Fig. 2). When the HCC cell lines were analyzed, only $\Delta 2$ GGCX was expressed in the DCP-positive HCC cell lines.

We further examined the function of $\Delta 2$ GGCX and demonstrated that transfection of a low proportion of $\Delta 2$ GGCX cDNA causes DCP production in DCP-negative HCC cell lines [50], suggesting that $\Delta 2$ GGCX could function as a dominant-negative inhibitor of wild type GGCX. It may be that the GGCX structure is altered in $\Delta 2$ GGCX, 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.

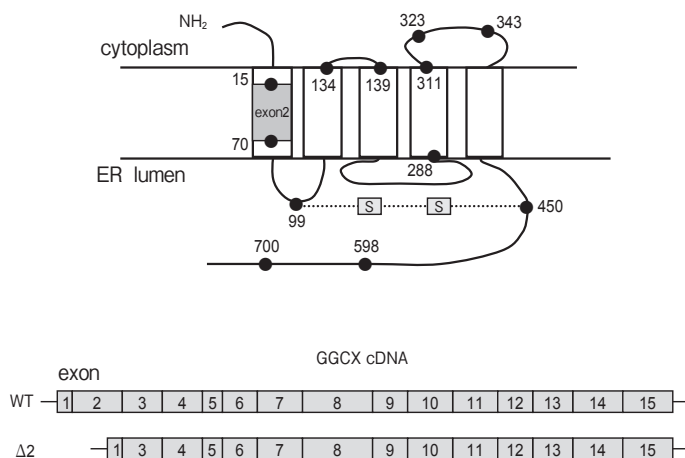


Fig. 2 A splicing variant of vitamin K-dependent γ -glutamyl carboxylase was identified in hepatocellular carcinoma cell lines. Top panel, structure of the vitamin K-dependent γ -glutamyl carboxylase (GGCX) transmembrane protein. Exon 2 is located in the first GGCX transmembrane domain. Bottom panel, 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 $\Delta 2$ GGCX 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.

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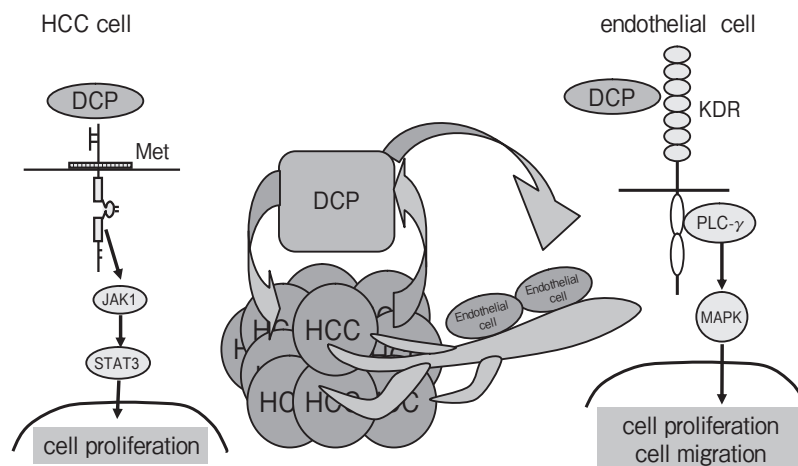


Fig. 3 Des- γ -carboxy prothrombin has the potential for both autocrine and paracrine stimulation in the development of hepatocellular carcinoma. Secreted des- γ -carboxy prothrombin (DCP) binds to Met on hepatocellular carcinoma (HCC) cells and stimulates the Met-Janus kinase 1-signal transducer and 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.

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