Review

Des-γ-carboxyprothrombin: Clinical effectiveness and biochemical importance

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Summary **Des-γ-carboxyprothrombin (DCP) is an abnormal prothrombin with a decreased number of γ-carboxylated glutamic acid residues in the Gla domain. DCP is also known to be an effective serological tumor marker for hepatocellular carcinoma (HCC), and highly sensitive methods of detecting serum DCP have enabled the detection of early and small-sized HCC in clinical settings. Several immunohistochemical studies have suggested that excessive production of DCP in HCC tissues may relate to worse tumor behavior such as the presence of vascular invasion and intrahepatic metastasis of HCC cells. Clinical availability of DCP, therefore, might be a more significant factor in the diagnosis of tumor behavior in HCC patients. Recently, some studies have suggested that DCP may play an important role in cancer progression *via* induction of cancer cell proliferation and angiogenesis around HCC tissues. Thus, DCP is expected to be effectively used not only as a tumor marker but also as a target of drug discovery.**

Keywords: Des-γ-carboxyprothrombin, Hepatocellular carcinoma, Tumor marker, Cancer cell proliferation, Angiogenesis

1. Introduction

Cancer cells are known to express various substances that are not expressed or expressed in small amounts, if at all, in normal cells. These cancer-related substances have been used as tumor markers in clinical settings. Various types of tumor markers have been discovered thus far and each tumor marker helps clinicians to diagnose a specific cancer disease in the early stage, to predict the cancer's behavior, and to determine a therapeutic strategy, thereby improving the survival of cancer patients (*1-3*).

Des-γ-carboxyprothrombin (DCP), also known as protein-induced vitamin K absence or antagonist II (PIVKA-II), is known to be an effective tumor marker for hepatocellular carcinoma (HCC) (*4-6*). Recent studies on DCP have revealed not only the effectiveness of this substance as a diagnostic marker but also its significant role in cancer progression. The present article reviews characteristics and clinical effectiveness of DCP as a diagnostic marker and describes further progression in the field of DCP investigation.

2. Mechanism of DCP Production

2.1. *Structural characteristics of DCP*

Prothrombin, a coagulation factor, is synthesized in a vitamin K-dependent manner in liver tissues. DCP is an abnormal prothrombin that lacks the ability to interact with other coagulation factors. The difference between normal prothrombin and DCP is the component of amino acid residues. The prothrombin molecule has some functional domain structures, and there are

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10 γ-carboxylated glutamic acid (Gla) residues in the N-terminal domain called the Gla domain (*7*). These Gla residues are originally glutamic acid (Glu) residues in the prothrombin precursor and are completely synthesized by vitamin K-dependent enzymatic reaction of γ-glutamyl carboxylase as post-translational modification (Figure 1) (*8*). When this reaction is insufficient under some conditions, such as a vitamin K deficiency, DCP with Glu residues in the Gla domain that do not undergo γ-carboxylation is expressed and secreted into extracellular regions (*9*). Thus, DCP comes in the form of many types of molecules with different numbers of Gla residues (*10*).

2.2. *Mechanism of DCP production*

The exact cause of DCP production in HCC tissues is not yet understood. One possibility is that the activity of γ-glutamyl carboxylase declines in HCC tissues. Shah *et al*. performed an analysis using rat models carrying a Morris hepatoma tumor to clarify the significance of γ-glutamyl carboxylase activity (*11*). Their study revealed that γ-glutamyl carboxylase activity was markedly lower in DCP-positive HCC tissues than in DCP-negative HCC tissues, while, abnormal γ-glutamyl carboxylase activity was not detected in normal liver tissues. Similar results were also obtained *via* an *in vitro* experiment using DCP-producing and non DCP-producing rat hepatoma cell lines (*12*). These studies indicated that an elevated DCP level may be a consequence of decreased γ-glutamyl carboxylase activity but not of abnormal prothrombin expression.

Another possibility is that the availability of vitamin K declines as a result of abnormal vitamin K metabolism. Okuda *et al*. performed a study using an HCC cell line to clarify the effect of vitamin K on the production of DCP and revealed that the HCC cell line produced DCP in a time- and cell number-dependent manner in the absence of vitamin K but not in the presence of vitamin K (*13*). Sakon *et al*. measured serum levels of DCP in patients with or without vitamin K administration and showed that the serum level of DCP declined as a result of administration of vitamin K while the levels of some vitamin K derivatives were significantly elevated in HCC patients (*14*), suggesting that elevated DCP may not be caused by vitamin K insufficiency but by abnormal vitamin K metabolism in HCC cells. In addition, Huisse *et al*. reported that a decreased vitamin K level and not decreased γ-glutamyl carboxylase expression was the most important factor for the production of DCP (*15*).

Furthermore, the overexpression of prothrombin precursor is also suggested to be one of causes of DCP production. Ono *et al*. indicated that the level of prothrombin precursor in HCC tissues was significantly elevated in patients with an elevated serum DCP level while there was no significant difference in the levels

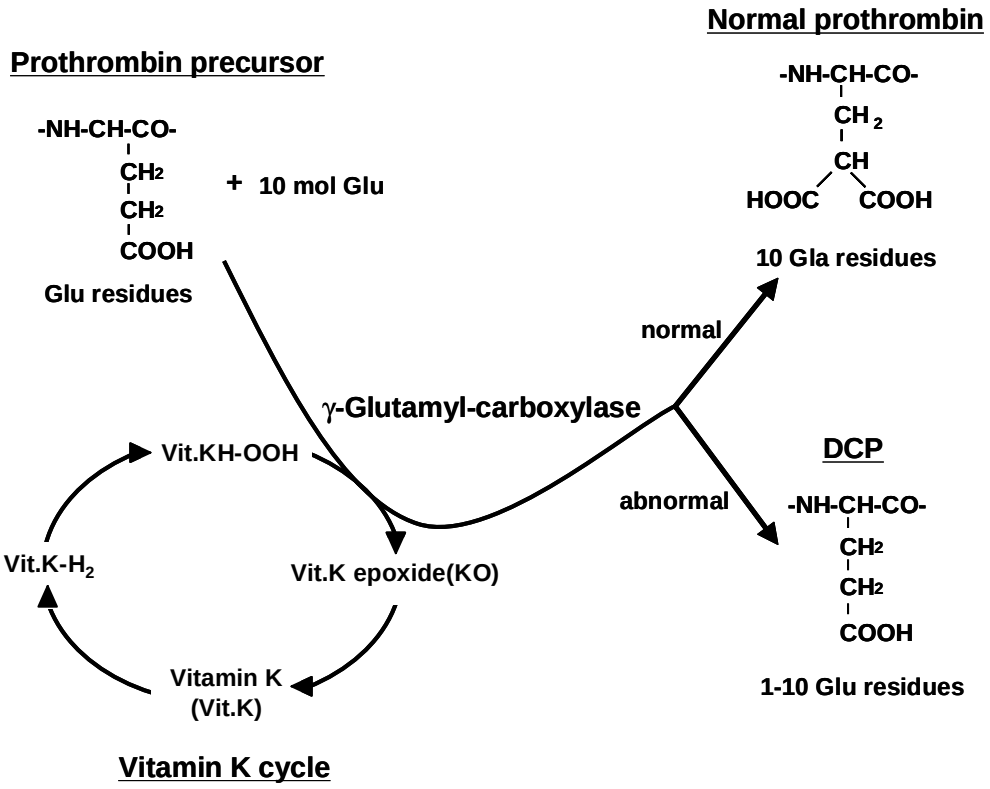

Figure 1. Production of normal prothrombin and DCP. Under normal conditions, γ-glutamyl-carboxylase completely alters 10 glutamic acid (Glu) residues in the Gla domain of a prothrombin precursor into 10 γ-carboxylated glutamic acid (Gla) residues. Under abnormal conditions, this reaction is insufficient, resulting in the production of an abnormal prothrombin, DCP, with a decreased number of Gla residues.

of endogenous vitamin K between HCC and non-HCC tissues (*16*). Yamagata *et al*. also suggested that the activity of γ-glutamyl carboxylase per unit amount of endogenous prothrombin precursor decreased in HCC tissues when compared to non-HCC liver tissues (*17*). These findings suggest that excessive production of prothrombin precursors may be a cause of DCP overexpression.

Production of prothrombin is affected by the expression and activity of various factors, so the mechanism of DCP production is also complicated. No single abnormal condition or phenomenon may cause DCP production but a combination of these conditions or phenomena may. In addition, these abnormalities are suggested to vary depending on the characteristics of HCC cell lines and cancer behavior in HCC patients. Further studies should be performed to clarify the mechanism of DCP production and evaluate its relationship to cancer behavior.

3. Clinical Effectiveness of DCP

3.1. *Development of tools for DCP detection*

As described above, DCP has been used as an effective tumor marker of HCC in clinical settings. In 1984, Liebman *et al*. first reported that the levels of DCP in sera of HCC patients were significantly elevated and that this elevation was related to recurrence of HCC after surgery (*18*). This study was performed by means of competitive radioimmunoassay with a polyclonal antibody to detect the level of DCP. Next, Fujiyama *et al*. determined plasma DCP levels using enzyme immunoassay (EIA) and reported that the levels of plasma DCP were frequently elevated in 63% of HCC patients with a cut-off level of 0.1 AU/mL; levels returned to normal after surgery in some patients while they rose again in cases of recurrence of the disease (*19,20*). These studies suggested that DCP could be used as a sensitive marker for HCC diagnosis and that the determination of DCP levels by EIA with monoclonal antibody is a powerful method of diagnosing HCC behavior easily and rapidly. However, the sensitivity of this method had to be increased to screen patients with a small-sized HCC.

An EIA kit with a higher sensitivity (Eitest PIVKA-II, Eisai, Tokyo, Japan) and an electrochemiluminescence kit (Picolumi PIVKA-II, Eisai) have been developed and are currently used in clinical settings. Their sensitivity is 10 times higher than that of the previous EIA kit thanks to modification of the EIA method or use of a new electrochemical method (*21,22*). Many investigations have been performed using these kits to clarify the usefulness of DCP as a diagnostic marker for HCC. Using this more sensitive EIA, Mita *et al*. analyzed serum DCP levels in 91 patients with HCC and 57 with cirrhosis and indicated that 56 of 91 HCC patients had positive DCP levels with a cut-off level of 40 mAU/mL while only 3 of 57 cirrhosis patients had positive DCP levels (*23*). Sensitivity and specificity of the test were estimated to be 62% and 95%, respectively, suggesting that determination of DCP levels by the sensitive EIA is a useful method for the early diagnosis of HCC. In addition, Nomura *et al*. and Sassa *et al*. indicated that the sensitive EIA has the potential to detect a small-sized HCC (*24,25*). These data accumulated using methods of measuring DCP levels with greater sensitivity have enabled clinicians to screen more patients with HCC, and DCP is now effectively used as a tumor marker for early detection of HCC in patients.

Anti-DCP monoclonal antibody has also been investigated and developed by some researchers (*26-28*). One anti-DCP monoclonal antibody called MU-3, which is now usually used to detect serum DCP levels in clinical settings, can recognize a specific part of the Gla domain as an epitope (*29*). Reaction of MU-3 is negatively dependent on the number of Gla residues in the DCP molecule. DCP variants produced in HCC patients chiefly have fewer than 4 Gla residues at the positions of amino acid residues 16, 25, 26, and 29 and are thereby strongly recognized by MU-3 (*10*). In contrast, DCP variants produced in patients with benign liver disease had more than 5 Gla residues. The kinds of DCP molecules which were detected in HCC patients were severely restricted, and MU-3 is a capable tool for screening HCC patients from patients with benign liver disease with a high level of specificity. Thus, using a sensitive test kit with MU-3 to measuring the serum level of DCP has significant clinical applicability.

3.2. *Clinicopathological significance of serum DCP levels*

Many studies on the relationship between the serum DCP level and various clinicopathological features of HCC have suggested that elevation of DCP reflects worse tumor behavior and prognosis for HCC patients (*30*). Imamura *et al*. indicated that patients' prognosis was significantly worse in patients with DCP-positive HCC than with DCP-negative HCC (*31*). Hamamura *et al*. reported that the positivity for DCP was frequently detected in patients with hepatitis B, a large tumor size, and enhanced tumor growth (*32*). Suehiro *et al*. also indicated that the serum level of DCP was related to the degree of proliferation of HCC tissues (*33*). Furthermore, several studies showed that elevated serum DCP is significantly related to portal vein invasion and/or intrahepatic metastasis as may be an independent prognostic factor. Sakon M *et al*. showed that a macroscopically massive carcinoma, intrahepatic metastasis, and portal vein tumor thrombus were detected at high frequencies in DCP-positive patients (*34*). Koike *et al*. performed a prospective study to clarify the significance of DCP in the prediction of portal vein invasion and revealed that portal vein invasion occurred at a significantly higher rate in patients who were positive

for DCP than those negative for DCP, and they suggested that positivity for DCP is the strongest predictive factor for portal vein invasion (*35*). Tang *et al*. also analyzed the clinicopathological significance of the serum level of DCP and indicated that a positive serum DCP level was significantly related to the presence of vascular invasion, intrahepatic metastasis, tumor size, TNM stage, and tumor recurrence (*36*). In addition, patients with positive serum DCP levels had a worse prognosis than those without such levels (*36*). Miyaaki *et al*. also indicated that DCP-positive HCC patients had higher frequencies of infiltrative growth, vascular invasion, and intrahepatic metastasis than DCP-negative HCC patients (*37*). These findings suggest that DCP is significant related to worse tumor behavior. Currently, the serum DCP level can be used not only as a diagnostic marker to screen HCC patients but also as an indicator of therapeutic effect and predictor of tumor recurrence and patient prognosis.

Other than DCP, there are two other serum markers for HCC diagnosis, α-fetoprotein (AFP) and the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) (*35*,*38-40*). Although elevated DCP in sera of HCC patients is suggested to have no relation to elevated AFP and AFP-L3, a simultaneous test with a combination of these tumor markers is more effective for sensitive diagnosis of HCC (*41*,*42*). Some studies showed that elevation of DCP detected in patients with low AFP levels was frequently indicative of a large tumor size (*32*,*35*). Nakamura *et al*. suggested that DCP is more effective for diagnosis of large tumors than small tumors while AFP is effective for diagnosis of small tumors (*39*). Thus, simultaneous measurement of DCP and AFP or AFP-L3 levels has been proposed as a way to screen patients with HCC and particularly as a way to detect a small-sized HCC with a high level of sensitivity.

3.3. *DCP expression in liver tissue and its relation to an elevated serum DCP level*

Since DCP in circulation is originally produced by HCC tissues, one can reasonably conclude that the level of DCP expression in tissues determines the serum DCP level. Actually, the level of DCP expression in HCC tissues correlates with the serum DCP level and is related to the biological malignant potential of HCC and the prognosis of small HCC (*43*). However, serum DCP in HCC patients may not originate solely from HCC tissues. Tang *et al*. showed that the overexpression of DCP is detected not only in HCC tissues but also in the surrounding non-HCC liver tissues and that patients presenting with an overexpression of DCP in the surrounding non-HCC tissues had significantly elevated serum DCP levels (*36*). In addition, Yuan *et al*. performed an electrochemiluminescence immunoassay to determine quantities of serum and tissue DCP and reported that levels of DCP expression in non-HCC tissues correlated with those in HCC tissues and serum DCP levels (*44*). These findings suggest that an elevated serum DCP level may originate not only in HCC tissues but in surrounding non-HCC tissues. Moreover, Tang W *et al*. performed a further study to clarify the clinical effectiveness of tissue and serum DCP levels (*45*). In analysis of patient survival, a patient group with both overexpressed tissue DCP and elevated serum DCP levels displayed the worst outcome when compared to other patient groups with negative tissue DCP expression and a low serum DCP level and with either overexpressed tissue DCP or an elevated serum DCP level. Furthermore, multivariate analysis identified DCP expression in total liver tissue as a significant prognostic factor along with intrahepatic metastasis. The current study showed that DCP expression in either HCC or surrounding liver tissue may be a powerful tool for diagnosing HCC behavior along with the serum DCP level (Figure 2). Moreover, the overexpression of DCP not only in HCC tissue but also in surrounding non-cancerous liver tissue must be considered in order to investigate the biological mechanism of an elevated DCP level in HCC patients

4. Biochemical Significance of DCP and Further Progression of Investigation

Although numerous studies have contributed to the recognition that DCP has a clinical significance as a useful diagnostic marker for HCC, reasons why DCP is overexpressed in HCC tissues and why this overexpression is related to enhancement of tumor growth and malignancy of HCC have not been clarified thus far. However, several basic studies on the function of DCP in HCC cells may offer important clues to resolving these questions.

4.1. *DCP's role in the proliferation of HCC cells*

The structure of DCP contains two kringle domains that are similar to those of hepatocyte growth factor (HGF) (*7*,*46*). The kringle domains are necessary for HGF to bind its receptor, called Met, and to induce cell proliferation. DCP is hypothesized to have the ability to bind with Met and cause cells, and particularly HCC cells, to proliferate. In 2005, Suzuki *et al*. investigated the biological function of DCP in HCC cell proliferation (*47*). According to their report, levels of DNA synthesis in HCC cell lines were significantly enhanced by addition of purified DCP; this enhancement was more marked in non-DCP-producing cell lines than in DCP-producing cell lines, indicating that DCP can induce the proliferation of HCC cells. Furthermore, the researchers also analyzed the mechanism of this phenomenon and revealed that DCP bound with Met and stimulated the Met-JAK-STAT pathway as the signaling pathway for the induction of HCC cell proliferation. These results suggest that DCP can induce the proliferation of HCC cells by functioning like HGF. DCP may relate to worse

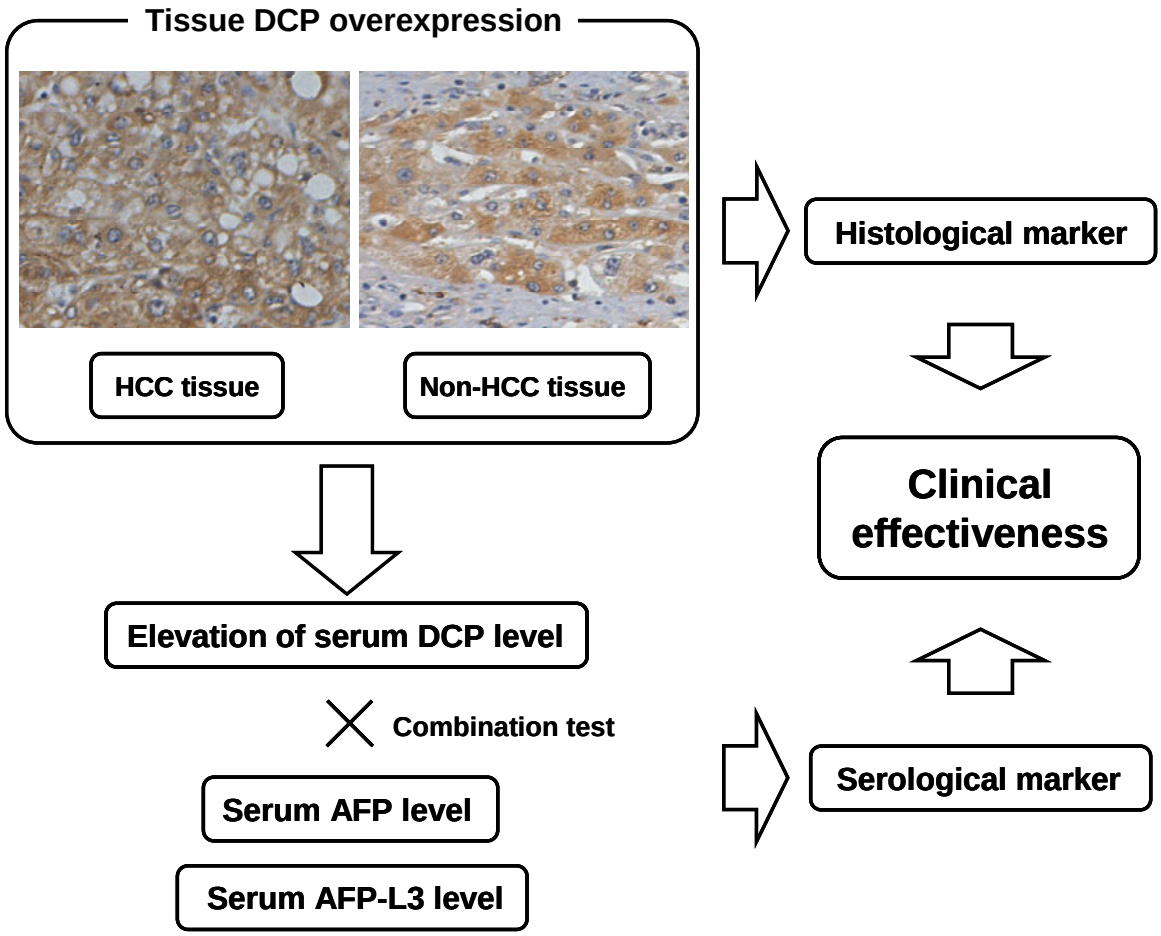

Figure 2. Clinical effectiveness of histochemical expression and serum level of DCP. Histochemical expression of DCP in both HCC and surrounding non-cancerous liver tissues is significantly related to worse tumor behavior. At the same time, the serum DCP level is also related to tumor malignancy; a combined serological test for DCP and AFP or AFP-L3 is particularly effective at diagnosing HCC in patients. Furthermore, the combination of histological and serological analyses may have novel clinical significance.

tumor behavior by enhancing the proliferation of HCC cells.

4.2. *DCP's role in angiogenesis around HCC tissue*

Another biological effect of DCP in malignancy of HCC may be that DCP has the ability to enhance angiogenesis around HCC tissues. Fujikawa *et al*. analyzed the biological function of DCP in angiogenesis by using human umbilical vein endothelial cells (HUVEC) (*48*). They indicated that DCP could stimulate the DNA synthesis and migrative activity of HUVEC but normal prothrombin could not. They reported that, as part of the activation of HUVEC, DCP could bind to kinase insert domain receptor (KDR), which is known to be a vascular endothelial growth factor receptor (VEGFR-2), and stimulate the KDR-PLC-γ-MAPK signaling pathway followed by the acceleration of DNA synthesis and cell migration (*48*). This fact suggests that DCP secreted from HCC cells can induce angiogenesis in surrounding tissues and cause a worse prognosis in HCC patients. Angiogenesis is a particularly important phenomenon for the continuous growth of tumor tissues and causes worse tumor behavior, such as dedifferentiation and hypervascularity (*49*); high tumor microvessel density is suggested to cause the recurrence of HCC (*50*). These studies suggest the significant role of DCP in cancer progression *via* the induction of angiogenesis and may helpful for understanding the mechanism by which DCP aggravates HCC behavior.

4.3. *Further progression of DCP investigation*

DCP has several important roles in HCC progression and may explain why cancer behavior and patient prognosis worsen in patients with DCP-positive HCC than in those with DCP-negative HCC. DCP is not just an abnormal prothrombin but may also be a potential cancer-enhancing protein (Figure 3). This fact suggests that DCP may be used not only as a diagnostic marker of HCC but also as a therapeutic target for HCC. As described above, DCP is related to some cancer-associated events such as self-proliferation and angiogenesis. Thus, if these events can be inhibited by some DCP inhibitor, progression of DCP-positive HCC may be suppressed. However, constructing novel DCP inhibitors will not be simple since the nature of DCP, such as its three-dimensional conformation, is not fully understood.

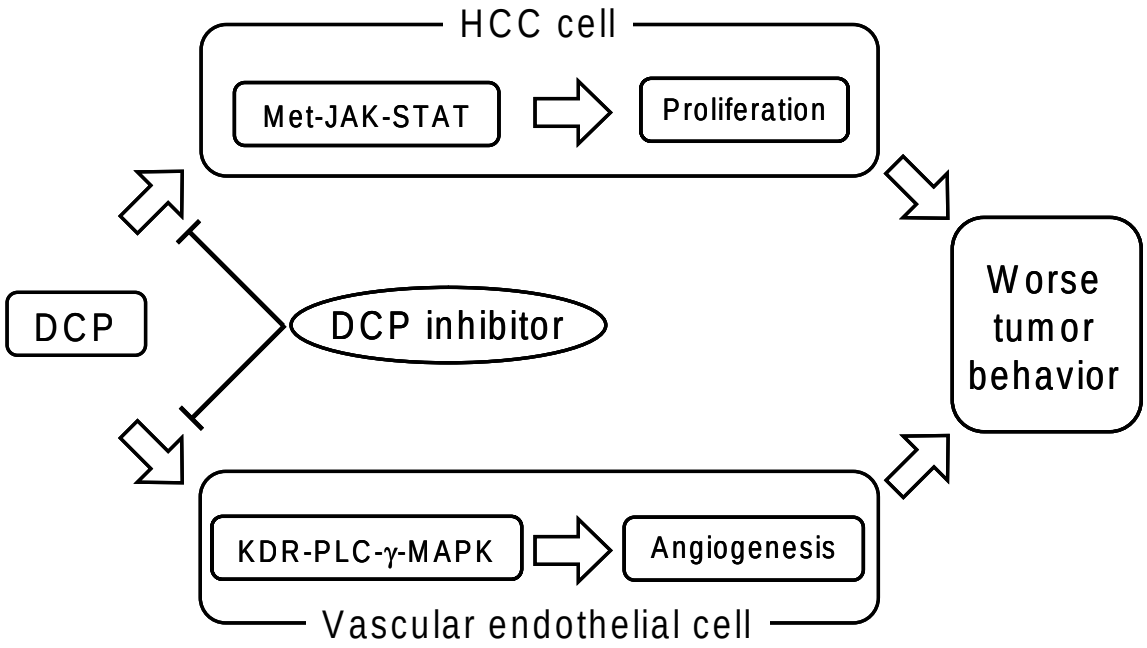

Figure 3. Proposed physiological functions of DCP and expected progression. Autocrine/paracrine secretion of DCP influences the self-proliferation of HCC cells through the Met-JAK-STAT signaling pathway. In addition, paracrine secretion of DCP influences the proliferation and migrative activity of vascular endothelial cells through the KDR-PLC-γ-MAPK signaling pathway. Secreted DCP can function as a growth factor, and hence the inhibition of DCP may contribute to the suppression of cancer aggressiveness.

Further investigation should be continued to develop the novel effectiveness of DCP.

5. Conclusions

In the current medical treatment for cancer, early diagnosis and appropriate therapeutics are the most important factors for the improved survival rate of cancer patients. This review has focused on the clinical effectiveness of DCP in the diagnosis and treatment of HCC. DCP is now established as an effective tumor marker for HCC and highly sensitive methods of detecting DCP contribute to the early diagnosis of HCC. In addition, recent findings indicating the novel biological functions of DCP in HCC progression may provide clues to novel therapeutics for HCC.

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