Do Soluble Vascular Endothelial Growth Factor and Its Receptors Predict the Progression of Chronic Hepatitis to Hepatocellular Carcinoma?

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Abstract

Angiogenesis occurs as a consequence of cellular hypoxic conditions. Occurring of chronic hepatocyte necro-inflammation, fibrosis, cellular proliferation, and infiltration of malignant cells may activate pro-angiogenic factors and induce the formation of new blood vessels. The closed relation between the angiogenesis factor genes mutation with its expression in the liver tissues and clinical appearances is poorly understood. In clinical practice, it is easier to measure angiogenesis soluble factors (ASF) than determine the tissue expression levels of pro-angiogenesis factors. Vascular endothelial growth factor (VEGF) is the most important regulator of angiogenesis. Soluble VEGF and VEGF receptor can be detected in blood (serum and plasma). Angiogenesis soluble factors are needed as the seromarker for facilitating the early detection of progressive chronic liver disease.

Introduction

Hepatocytes are exposed to hypoxic conditions during necro-inflammation, fibrosis, hepatocyte proliferation, infiltration of malignant cells, and metastasis. The mitogen-activated protein kinase (MAPK) signaling pathway (RAS/RAF/MEK/ERK) is activated by increasing of VEGF expression in the liver tissues. Genetic mutations may affect the VEGF and VEGF receptors (VEGFRs) expression in target tissues. DNA mutations and seromarkers of angiogenesis have been studied as potential predictors of survival and relapse in hepatocellular carcinoma subjects. The excess of VEGFRs secretion from endothelial cells plays an important role in blood vessel sprouting and neo-angiogenesis. However, it is poorly understood and few studies mentioned on its relationship with diseases such as preeclampsia, retinopathy and chronic kidney disease. How far neo-angiogenesis processes in chronic hepatitis and liver cirrhosis tend to favor the proliferation of malignant cells and relapse? Do the soluble VEGF and VEGFRs, as seromarkers, predict tumor relapse? This review will discuss the use of angiogenesis soluble factors (VEGF and VEGFRs) as seromarkers of predictor progression in chronic hepatitis to hepatocellular carcinoma (HCC).

Roles of VEGF and VEGFRs in chronic hepatitis and hepatocellular carcinoma

VEGF is a polypeptide, belonging to the platelet-derived growth factor (PDGF family of growth factors. These growth factors consist of dimeric cysteine-linked chains that are secreted as glycoproteins with a molecular weight of about 40 kDa. VEGF is encoded by a family of genes, including VEGF-A, -B, -C, -D and placental growth factor (PIGF). Human VEGF is most commonly expressed by the VEGF-A gene [1].

VEGF is produced and activated in the proliferation of endothelial cells. Its regulation of the vascular permeability of endothelial cells in the liver sinusoid involves increased expression of VEGFR-1 in hepatocytes and stellate cells, as well as increased expression of VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1) in hepatocytes and sinusoid endothelial cells. Binding of VEGF to VEGFR-1 on hepatocytes stimulates autocrine-mediated proliferation of hepatocytes. Hepatocyte proliferation is also mediated by the paracrine activities of hepatocyte growth factor (HGF) and interleukin (IL)-6, which are expressed by sinusoidal endothelial cells. Activation of VEGFR-2 stimulates the proliferation of sinusoidal cells. Over expression of VEGF is associated with increased of

microvasculature formation and metastasis, but decrease in apoptosis spontaneously. VEGFR-2 (Flk-1) is one of the main mediators of the mitogenic and angiogenic processes occurring in cell transformation and tumorigenesis [2]. High expression of VEGFRs liver tissue correlates with clinical appearance (hepatocytes inflammation, fibrosis and cirrhotic) and etiology (hepatitis B virus) [3,4].

Excessive expression of tyrosine kinase receptors (TKRs) owing to the activation of mutations or excessive expression of their ligands (e.g., VEGF, PDGF, and epidermal growth factor [EGF]) affects the downstream signaling pathways. VEGFR-2 plays dominant roles in the proliferation of endothelial cells, cell migration, vascular permeability, and cell survival (decreased apoptosis) through three pathways: the heat shock protein or stress response signaling pathway, the Wnt pathway, and the MAPK (RAS/RAF/MEK/ERK) signaling pathway. VEGFR-1 plays a role in vasculogenesis and VEGFR-3 plays a role in lymphogenesis. The MAPK pathway (RAS/RAF/MEK/ERK) is an important signaling pathway involved in the proliferation of tumor, differentiation, and apoptosis cells. The interactions between VEGF signaling and stimulation of the MAPK pathway (RAS/RAF/MEK/ERK) in angiogenesis, proliferation, and metastasis are illustrated in **(Figure 1)** [5].

Besides stimulating of sinusoidal endothelial cells proliferation, VEGF also stimulates the expression of protease-like collagenase, matrix metalloproteinase, urokinase, and tissue-type plasminogen activators. The activated sinusoidal endothelial cells may damage the surrounding extracellular matrix to facilitate their migration and then form new blood vessels. Other factors involved in the regulation of angiogenesis include members of the angiopoietin (Ang) and Tie families, particularly Ang-1, Ang-2, and the TKRs Tie-

Figure 1 Interaction between VEGF, VEGFR-2, and sVEGFR-2 via MAPK pathway (RAS/RAF/MEK/ERK signaling pathway). The mitogen-activated protein kinase signaling pathway (RAS/RAF/MEK/ERK) plays important roles in the proliferation, differentiation, and apoptosis of malignant cells. PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor [2].

1 and Tie-2. The mechanisms of involving in the regeneration of liver tissue in HCC include sprouting angiogenesis, intussuscepted angiogenesis, vasculogenesis, vasculogenic mimicry, vessel co-option, and lymph–angiogenesis are illustrated in **(Figure 2)** [2].

Phenotypic changes in the DNA repair genes in liver cirrhosis and fibrosis during active inflammation are associated with progression to HCC. Hepatitis B and C virus may promote hepato-carcinogenesis directly and indirectly via accumulation of intra cellular genetic mutations, but it is still unclear. The points mutation are usually insertion or deletion at proto-oncogene and tumor suppressor gene, these can influence to mismatch repair gene in DNA host [6].

HBX-protein of hepatitis B virus (HBV) directly induces hepatocytes carcinogenesis. The exon of retinoic acid receptor B and cyclin A2 genes of HBV may integrate in proto-oncogenes sequence regulator host, without disturbed to tumor suppressor genes [6-9]. Indirectly host integrated of HBV in is caused by chronic hepatocytes necro-inflammation, necrosis, and regeneration. Hepatocytes infected with HBV bearing pre-S mutations ("ground glass hepatocytes") which contained of pre-S mutants' deletion in the endoplasmic reticulum can inhibit the cell's physiologic activities and activate VEGF-A expression in liver tissues. Because of rapid cells regeneration, hepatocytes contained fixed specific HBV DNA gene will be owned in other hepatocytes ("daughter cells"). These cells may also be changed to pre-neoplastic cells that have the high risk of HCC [6-10].

Hepatitis C virus (HCV) has not integrated in hepatocytes, but HCV-core protein directly induce hepatocytes to carcinoma cells, in spite of no sign of chronic hepatitis and cirrhosis, clinically. Mechanism of HCV gene integrated in human genome and promoted carcinogenesis, is similar with HBV. Proto-oncogenes and growth-regulator genes are the integration sites that take a role in tumorigenesis [11]. Hepatitis C virus can stimulate the activity of the VEGF gene promoter by stabilizing the activity of hypoxia-inducible factor-1, and inducing the expression and secretion of the VEGF-A product [12].

Liver fibrosis is a common event in chronic liver disease and cirrhosis because of the increased activity of growth factors (e.g., VEGF, α fibroblast growth factor [FGF], and βFGF) produced by hepatocytes, and hyper-dynamic activity of the vasculature caused by portal hypertension. Increases in the expression of VEGF, VEGFR-2, and CD31, an endothelial cell marker of tissue fibrosis, coupled with portal hypertension greatly increase the risk of malignancy [13,14].

Polymorphisms of VEGF gene can influence to expression of VEGF and VEGFR. Most of VEGF gene polymorphisms are located at promoter, and few at 5'UTR, intron and 3'UTR. There is no polymorphism located at coding region. Previous study revealed that there are association between VEGF gene polymorphisms with hyper expression of VEGF and VEGFRs in liver tissue and blood (serum and plasma). VEGF gene polymorphisms are also associated with clinico-pathologic, recurrence after surgery and mortality rate. Although differ in location, genotype and allele prevalence [15,16].
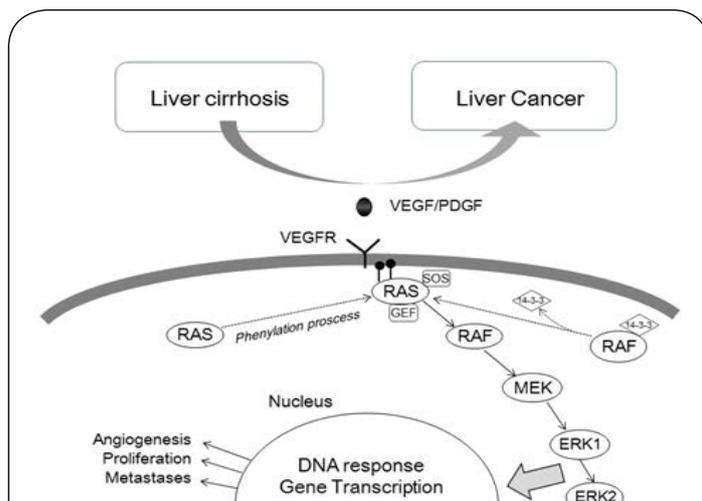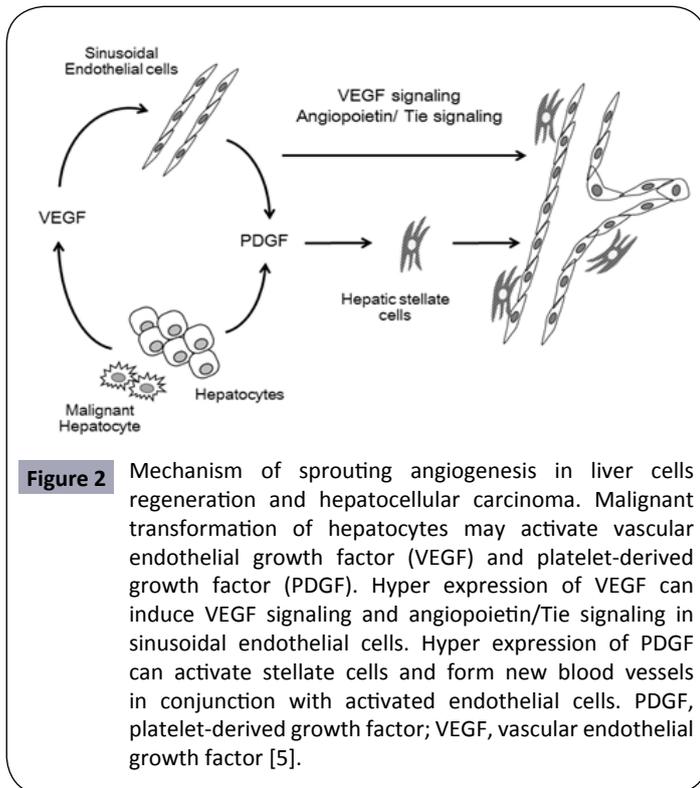
Figure 2 Mechanism of sprouting angiogenesis in liver cells regeneration and hepatocellular carcinoma. Malignant transformation of hepatocytes may activate vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Hyper expression of VEGF can induce VEGF signaling and angiopoietin/Tie signaling in sinusoidal endothelial cells. Hyper expression of PDGF can activate stellate cells and form new blood vessels in conjunction with activated endothelial cells. PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor [5].
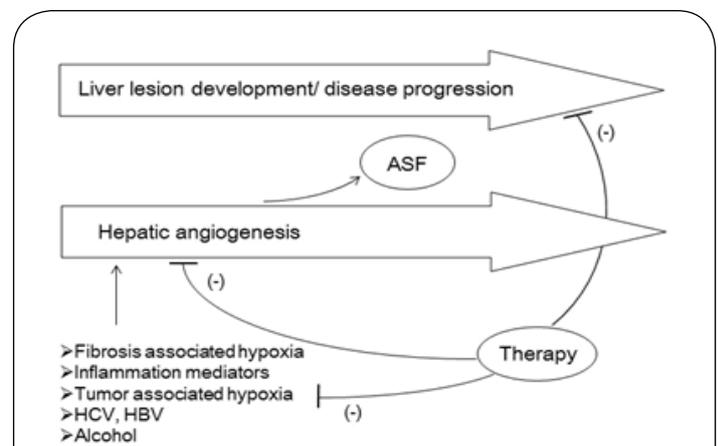
Angiogenesis Soluble Factors (ASF)

The presence of ASF in plasma and serum is now accepted as a sign of angiogenesis in liver disease. The development of lesions in hepatocytes is a stage of angiogenesis, and the presence of ASF is a critical sign linking both processes **(Figure 3)**. Source cells (SCs) in the liver differentiate into a variety of cell types, including hepatocytes, Kupffer cells, infiltrating inflammatory cells, stellate cells, endothelial cells, and epithelial cells. Pro-angiogenic factor activates SCs and surrounding target cells (TCs), including endothelial cells, pericytes, and epithelial cells. The signal of ASF between the SCs and TCs can reach the systemic circulation, and be detected in blood (serum and plasma) **(Figure 4)** [17].

VEGF and sVEGFR-2 are examples of ASF that have been used as seromarkers for evaluating the efficacy of anti-VEGF therapies, in which an increased serum VEGF concentration is followed by a decreased sVEGFR-2 concentration, and the circulating sVEGFR-2 concentration is highly correlated with its expression in the liver [17-19]. The serum VEGF concentrations in HCC and acute hepatitis patients are higher than those in healthy or cirrhosis, but the serum VEGF concentration in cirrhosis patients is lower than that in healthy [17,20]. The serum VEGF and sVEGFR in HCC patients can predict the overall survival and prognosis, and also be used as biomarkers of anti-angiogenesis therapy and predicting HCC relapse [19-21].
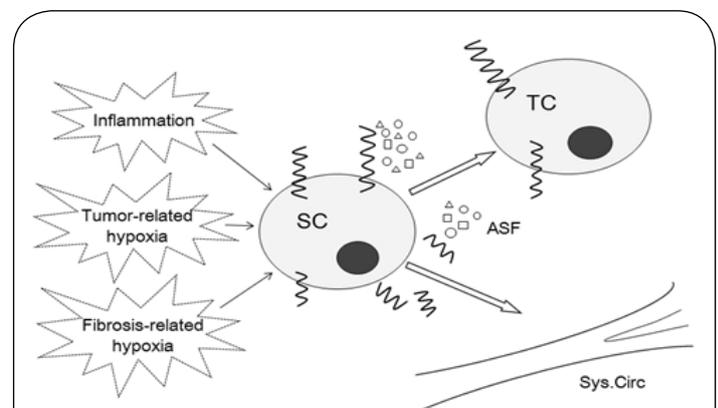
Soluble VEGFR-2 shows greater potential as a biomarker than does in VEGF, because many factors affect the serum VEGF concentration, such as: aggregation and non-aggregation of thrombocytes, leukocyte activity, the release of cytokines, extracellular matrix components, fibronectin, fibrin, and thrombospondin-1. Accordingly, the VEGF concentration in serum differs from that in plasma, whereas the sVEGFR-2 concentration does not differ markedly between serum and plasma [18-21].

To date, few studies have studied the potential of serum or plasma VEGF and sVEGFR-2 concentrations as seromarkers for the progression of chronic liver diseases (e.g., hepatitis B, hepatitis C, and cirrhosis) to HCC. Additionally, based on our knowledge and web journal searching, there are few studies have mentioned on the relationship between the VEGF gene mutations, liver tissue expression of VEGF and VEGFRs, and the soluble VEGF and VEGFRs in chronic hepatitis and/or cirrhosis. Therefore, future research should examine whether seromarkers of angiogenesis and VEGF gene mutations can predict disease progression and survival in chronic liver diseases.

Figure 3 The potential of angiogenesis soluble factors (ASF) as biomarker of angiogenesis. The development of lesions in hepatocytes is an important stage in angiogenesis, and the presence of angiogenesis soluble factors (ASF) is a critical sign of both processes. The presence of ASF in plasma or serum is indicative of ongoing angiogenesis in liver disease. ASF, angiogenesis soluble factors; HBV, hepatitis B virus; HCV, hepatitis C virus [17].

Figure 4 The dynamic of angiogenesis soluble factors (ASF). Proangiogenic factors target source cells and surrounding target cells, including endothelial cells, pericyte, and epithelial cells. The angiogenesis soluble factors can reach the systemic circulation and can be detected in serum. ASF, angiogenesis soluble factors; Sys.Circ, systemic circulation; TC, target cells; SC, source cells [17].

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