What is the role of Von Willebrand factor in chronic hepatitis B virus infection to hepatocellular carcinoma: a review article

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Abstract: Von Willebrand factor (VWF) is a glycoprotein synthesized and secreted by vascular endothelial cells and megakaryocytes, found on plasma surface, endothelial cells, and α-granule of platelets. VWF can be interacted with collagen and platelet membrane glycoproteins GPIb and GPIb-IIa and play an important role in platelet adhesion and aggregation. Growing research evidence suggests that VWF also mediates the prevention or protesting of hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB) patients from several clinical studies. While the mechanism of VWF in HCC protection or protest is still unclear, further study is required. This article aims to rationalize the role of VWF in the development of HCC, and the functional domain of VWF in cancer as well as cross-talking with platelets and miRNAs. This article also looks forward to the future development and challenges of VWF research.

Keywords: chronic HBV infection, HCC, hemostasis, VWF

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Introduction
Von Willebrand factor (VWF), well known for its function in normal and causes bleeding disorder due to Von Willebrand disease (VWD), produced by the endothelial cells (ECs) and abundantly stored in α-granules of platelets, has important role in coagulation and hemostasis. Insufficient VWF or functional defect can produce growth time coagulation and hemostasis, which is related to coagulation diseases such as cancer and also related to inflammatory, metabolic, cardiovascular, and neurodegenerative diseases. VWF has a complex multidomain structure that can interact with a variety of types of cells, including collagen, GPIB/IX/V complex GPIb/IIa, and TSP-1, an intrinsic component of the subendothelial matrix; A Disintegrin And Metalloproteinase (ADAMTS) family of metalloproteinases with a thrombospondin type 1 motif and expressed predominantly by liver. The plasma VWF combine with fibrinogen and other factors. It absorbs more platelets and makes platelet thrombosis progress. On the contrary, in ECs, VWF is secreted or stored in Weibel–Palade body and released after stimulation. The differences of endothelial- and platelet-derived VWF are shown in Table 1. VWF and recently discovered TSP-1 have a critical role in platelet adhesion to endothelium under high shear condition: platelet glycoprotein GPIb binds to subendothelial VWF or TSP-1; this mediates the adhesion of platelets to collagen and other secondary endothelial molecules, the release of platelet granule content and the activation of platelet αIIbb3 integrin. Binding of adhesion proteins, such as fibrinogen and VWF, activates αIIb3, allowing for the collection of more platelets to form thrombosis. Platelets are related to tumor progression and play a key role in hepatocellular carcinoma (HCC) (Pavlovic et al.) Increasing evidence from both basic and clinic studies suggests that VWF promotes chronic hepatitis B virus (HBV)- or hepatitis C virus (HCV)-associated HCC. However, the mechanism of VWF in the development of HCC needs further study. In this article, the role of VWF in the development of HCC and its interaction with platelets, as well as miRNA were also reviewed.
Structure of VWF

VWF gene is located on the short arm of chromosome 12. The full-length cDNA of VWF is 8900 bp. The main translation products consist of a 22 amino acids signal peptide and a large 741 amino acids pre-peptide sequence. The function of vWF residues after signal peptide is unknown, which may be related to the formation of polymers. Interestingly, it also contains an Arg-gly-Asp (RGD) sequence that also exists in the GPIIb-IIIa protein.

Mature VWF contains five different domains, totaling 2050 amino acids. One domain contains 193–2, 20 amino acid residues, three consecutive copies, named as A1, A2, and A3, located in 497–1111 peptides. Domain B contains 25–35 amino acids with two copies, located in 1533–1636 peptides. The C domains contain 116–119 amino acid residues, with two copies, located at 1637–1899 period. Total VWF of peptide segments as shown in Figure 1.

Both domains B and D contain abundant cys residues, which can promote disulfide dimerization stably. In addition, some glycosylation sites in these two regions bind to glycoproteins and are involved in platelet activation, adhesion, and aggregation. The distribution of molecular cys residues is similar to FVIII and FV, mainly concentrated in the both sides of the molecule. There are only six cys residues in A domain, while the two sides: B domain and D domain, contain 163 cys residues. VWF F monomer is transformed into dimer by stable disulfide bond at segment C. The dimer monomer formed by weak disulfide bond at the N end is the structural basis of its function, while the polymer is just a replicator with the same function and improves its function at the same time. The C domain appears in the C-terminal region (CTR), which also contains RGD motifs responsible for the interaction between endorphin cells and tumor cells.

VWF regulation

Several molecular-level evidence proposes different mechanisms of VWF activation. However, the exact mechanism of VWF function remains to be further studied. VWF plays a major role in platelet-related diseases, so it is considered as a therapeutic target for anti-thrombotic therapy. In contrast, sustained activation of VWF can have harmful effects on different types of cancer cells. This dual function of VWF raises the question of whether it is necessary to activate or inhibit VWF in cancer treatment of patients with TTP. This contradictory function of VWF leads to the debate about whether VWF is a curse or a blessing in cancer treatment. ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). It was found that the interaction with the A3 domain of VWF may lead to competition with TSP-1, which slows the rate of VWF proteolysis.

Table 1. The comparison of endothelial- and platelet-derived VWF.

| Endothelial-derived VWF | Platelet-derived VWF |
|-------------------------|----------------------|
| Storage                 | Weibel–Palade bodies | α-granules           |
| Secetion                | Constitution         | Non-constitution     |
| High molecular weight multimers | Weibel–Palade bodies | α-granules           |
| Protein binding         | Collagen             | Collagen             |
| Protein expression      | Sialic acid          | Sialic acid          |
|                         | Galactose            | Galactose            |
|                         | ABO antigen          |                      |

VWF, Von Willebrand factor.
C-terminal of TSP-1 bind to the disulfide bond between VWF dimer. Meanwhile, VWF can also be regulated by phosphorylation. There are 22 glycosylation sites in VWF monomer, including 12 ASPs and 10 Thrs, which is similar to the distribution of Cysteine residues, most of them are at both ends of the molecule, and there is a large region between the peptide 916–1469, of which 8 of the Thr glycosylation sites are located in the peptide. The general concept of abnormal glycosylation expressed in glycophospholipids and glycoproteins is considered to be an important mechanism determining the stage, direction, and fate of tumor progression. In addition, Ward et al. has reported that the asialoglycoprotein receptor (ASGPR), which is one type of lectin receptors and expressed predominantly on hepatocytes; and preferentially binds to the glycosylation sites typically presented on VWF, plays a role in VWF clearance.

Function of VWF in tumorigenesis, in HCC invasion and metastasis

Tumorigenesis is potentially initial from angiogenesis, which is the process that blood vessels have renewed from the pre-existing ones. It is required for tumor growth and metastasis and also play a key role in the process of HCC development. The research studies from Starke et al. showed that VWF is participating in blood vessel formation and also as a negative regulator of angiogenesis. To date, it is clear that VWF plays multiple roles in the vascular system. As shown above, the huge and multiple structure of VWF supports several cell surface receptors and extracellular matrix proteins interactions and communication. VWF exists in three kinds of cells: ECs and megakaryocytes (MC); plasma (mainly from EC release) and subendothelial (via EC release), shown in Table 1. contains a number of molecules which have been involved in angiogenesis. Thomas and Augustin have reported the role of the angiopoietins in vascular morphogenesis in their studies; some other researchers also have identified novel molecular as component of Weibel–Palade bodies from ECs.

Besides associated with angiogenesis, tumor growth and metastasis, VWF also linked to the inflammation process from the evidence based on different inflammatory animal models; for example, Denis et al. have reported that the impaired
P-selectin surface expression and subsequent defects in leukocyte recruitment in the early phases of inflammation were observed in deficient VWF mice. VWF-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke, which have been concluded by the study from Zhao et al.;34 Meanwhile Methia et al.35 have observed that localized reduction of atherosclerosis in VWF-deficient mice and also VWF influences blood–brain barrier permeability and brain inflammation based on the experimental allergic encephalomyelitis animal model.36 However, this association has not been well confirmed in patients, which may be attributed to the multifactorial nature of these inflammatory conditions.

On the contrary, much more evidence suggests that reduction of angiogenic response in late passage may not be the result of the potential progressive reduction of VWF. On the contrary, it may be related to the decreased expression of VE cadherin, vascular endothelial growth factor (VEGF), galectin-3 or avb3.37–40 Guan et al.41 reported that the development of HCC is associated with VEGF, since the level of vascular endothelial growth factor, metastasis, and tumor volume in HCC-patients are higher than those in non-HCC patients.

In addition to the function of VWF targeting genes, these genes also play a typical role in tumor genesis and cell proliferation in a variety of cancers including HCC. Angiopoietin 2 (Ang2) is produced and stored in the Weibel–Palade storage granules of ECs, which is also believed to promote the stimulation of VEGF-dependent EC germination and migration.42

Above all, VWF may affect many important pathways involved in angiogenesis and vascular stability. Till now, increasing evidence suggests that Notch has an attractive role in liver development and tumor angiogenesis.43 In mouse models, activated Notch signaling has been shown to promote the formation of liver tumors.44 Furthermore, up-regulated of Notch1 signaling can increase the carcinogenic potential of human HCC cells.45 Interestingly, some available data suggest that the role of Notch signaling in HCC is controversial. Qi et al.46 reported that Notch signal plays an anti-tumor role in HCC. In agreement with the finding, the up-regulation of p53 by Notch1 could enhance HCC cells to sensitive to tumor necrosis factor (TNF)-related apoptosis.47 Also, in HCC, Viatour et al.48 reported that Notch activity was specifically up-regulated by retinoblastoma (RB) pathway could reduce cell proliferation and tumor growth. Therefore, considering the involvement of Notch signaling in HCC, targeting the Notch pathway might provide a valid strategy for HCC therapy.

Generally, more than 90% of deaths from solid tumors are attributable to tumor metastasis.49 Although the medicine and technology of HCC treatment have been developed and improved, the high rate of hepatic metastasis resulting in the poor survival in the end.50 VWF plays a key role in tumor development and is related to tumor metastasis. Terraube et al.51 suggested that VWF have played protective role against tumor cells in lung. VWF initial display inducing tumor cell apoptosis within a short time.52 In addition, after the recovery of VWF antibody, the number of metastatic tumors in the knockout mice was lower than that in the wild-type mice.53 Zanetto and colleagues have also found that the higher level of VWF and significantly increased platelet function in patients with HCC as well as patients in cirrhosis, especially.54 Cirrhosis and HCC are a continuum, the platelet aggregation is significantly increased and particularly in patients who have already suffered some cirrhosis-related complications, and it is associated with the risk of cirrhosis progressing to further complications and death. All the above findings pave the way for further studies to clarify the inhibition of platelets-VWF, which could be beneficial for patients with liver cirrhosis and HCC.55

**VWF crosstalk platelets and microRNAs in HCC**

Platelets are small, nuclear-free, disk-like blood components produced by MCs in bone marrow. The critical role of platelets in hemostasis is to form blood clots after activation and adherence to ECM in the vascular damaged sites.56 VWF has functional binding domains with platelet glycoprotein Ib, glycoprotein IIb/IIIa, collagen and heparin and served a crucial function in hemostasis and inducible activation, adhesion, and aggregation of platelets.57 Yun et al.56 found that platelets are activated and recruited in the liver after organ damage, and it plays an important role in tissue regeneration, mainly by secreting high concentration of serotonin and promoting hepatocyte proliferation. In addition to the potential
direct effect on HCC cells, platelets also interact with several different types of cells in the matrix, such as hepatic stellate cells, ECs and some hepatic immune cells.\textsuperscript{58,59} In this tumor matrix interaction microenvironment, platelet-derived factors directly affect tumor cell proliferation, fibrinogen signaling and immune cell recruitment, and contribute to more invasive and metastatic role in liver cancer.\textsuperscript{60–62} In addition, VWF is present in ECs and subendothelium, participating and implicating in the pathophysiological processes of HCC. Similarly, VWF deficiency induces survival of tumor cell in lung and results in more metastasis nodules.\textsuperscript{63} On the other side, reduced cellular apoptosis were observed in VWF-deficient group animals. Furthermore, anti-platelet pharmacological agents, such as ADP, aspirin, have been used in anti-tumor clinical trials.\textsuperscript{64}

Recently, several studies reported that a significant correlation of miRNAs in normal and pathological conditions.\textsuperscript{65} In particular, many miRNAs were found to be regulators of multiple types of HCC cell lines.\textsuperscript{66–69} In addition, miRNA can also be used as epigenetic regulator of gene expression to control many aspects of liver metastasis.\textsuperscript{70,71} MiRNA regulation can participate in multiple stages of cancer cell extravasation from the primary site, invasion, survival and growth in distant sites.\textsuperscript{72–74} New evidence shows that miRNA plays an important role in regulating the signal pathway of vWF. Integrated bioinformatics analysis reported that VWF as a key candidate in miRNA-mRNA regulatory axes, which is participated in the modulation of colorectal cancer (CRC) liver metastasis.\textsuperscript{75,76} Similarly, VWF rescued by miR-24 inhibition that induced osteosarcoma cells proliferation and migration.\textsuperscript{77} Since there is no effective targeted therapy to prevent or prevent liver cancer, miRNAs may be a potential new treatment.

**Future prospects of VWF in HCC research**

To be noted, undeniable progress has been made over the past few decades in our understanding of the structure and function of VWF. Several nonspecific VWF antagonists have been identified recently, including Heparin,\textsuperscript{78} Statins,\textsuperscript{79} N-Acetylcysteine (NAC),\textsuperscript{80} Aurintricarboxylic acid (ATA),\textsuperscript{81} Corticosteroids,\textsuperscript{82} Anti-TNF\textsubscript{α},\textsuperscript{83} and Colchicine\textsuperscript{84} specific VWF antagonists to inhibit VWF-GP\textsubscript{Ib} interaction, such as h6B4-Fab, GPG-290, AJvW-2,\textsuperscript{85–87} AJW200\textsuperscript{88,89} inhibits VWF-collagen interaction, such as 82D6A3, SZ-123\textsuperscript{90,91} And ALX-0081 (capracizumab), nanoparticles inhibit VWF GPI\textsubscript{Ib} interaction;\textsuperscript{92} VWF recombinant fragment VCL,\textsuperscript{93} recombinant ADAMTS13 (radamts13),\textsuperscript{94} Therefore, VWF inhibition or VWF-targeted combinations as well as some regional approaches, such as hepatic arterial perfusion, can be tested as improved treatment for HCC therapy.

**Conclusion**

As we all know, cancer is usually a disease driven by a variety of genetic abnormalities. The etiology of HCC varies from patient to patient, as similarly as the molecular proliferation of all patients. The overview of VWF in HCC are shown in Table 2 and Figure 2. In fact, many aspects of VWF biology need to be further studied, especially its cellular signal transduction pathways and also viral etiology in human physiology before it can be used as a therapeutic target.

**Table 2.** The summary of main studies on VWF, platelet and HCC.

| Description                   | References                                |
|-------------------------------|-------------------------------------------|
| Regulation                    | Da et al.\textsuperscript{24}             |
| Tumorigenesis                 | Haibe et al.\textsuperscript{26}          |
|                               | Hilmi et al.\textsuperscript{27}          |
|                               | Thomas et al.\textsuperscript{30}         |
| Invasion and metastasis       | Methia et al.\textsuperscript{35}         |
|                               | Hodivala-Dilke\textsuperscript{40}        |
|                               | Guan et al.\textsuperscript{41}           |
|                               | Felcht et al.\textsuperscript{42}         |
|                               | Villanueva et al.\textsuperscript{44}     |
|                               | Viator et al.\textsuperscript{48}         |
|                               | Valastyan et al.\textsuperscript{49}      |
| Cross talking                 | ReFAravalli et al.\textsuperscript{50}    |
|                               | Terraube et al.\textsuperscript{52}       |
|                               | Shavit et al.\textsuperscript{53}         |
|                               | Zanetto et al.\textsuperscript{54}        |
|                               | Zanetto et al.\textsuperscript{55}        |
|                               | Yun et al.\textsuperscript{56}            |
|                               | Sitia et al.\textsuperscript{40}          |
|                               | Sitia et al.\textsuperscript{61}          |
|                               | Carr et al.\textsuperscript{62}           |
|                               | Oleksowicz et al.\textsuperscript{63}     |
|                               | Mahmoudian-Sani et al.\textsuperscript{69}|
|                               | Lou et al.\textsuperscript{70}            |

VWF, Von Willebrand factor; HCC, hepatocellular carcinoma.
Figure 2. The overview of VWF in HCC is shown.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions
Qiong Xiang: Conceptualization; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Jia-Sheng Tao: Methodology; Validation; Writing – original draft; Writing – review & editing.

Jing-Jing Li: Investigation; Methodology; Writing – review & editing.

Rong-Bo Tian: Validation; Visualization; Writing – review & editing.

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