

The Potential Role of SEPT6 in Liver Fibrosis and Human Hepatocellular Carcinoma

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Liver fibrosis is a reversible wound-healing response in which a variety of cells and factors are involved in and results in excessive deposition of extracellular matrix (ECM) [1]. Cirrhosis is one of the significant causes of portal hypertension and end-stage liver disease, and it is the 14th most common cause of death around the world. Approximately 1.03 million people worldwide die from liver cirrhosis every year [2]. If patients with chronic liver disease are not intervened promptly, they will progress into liver fibrosis and cirrhosis within 5 to 10 years. In this pathological process, the decrease in the number of normally functioning hepatocytes, changes in the structure and function of liver lobules, and disordered blood circulation could all lead to the gradual loss of the normal physiological function of the liver. Liver fibrosis is regarded as a precancerous lesion [3], and patients have a high risk of developing into hepatocellular carcinoma (HCC) [4]. More than one million patients develop from progressive liver fibrosis to cirrhosis leading to death every year [5].

Hepatic stellate cells (HSCs) are a type of peri-sinus cells that are distributed throughout the liver. In normal liver, HSCs participate in the storage of retinoic acid, regulate blood vessels through endothelial cell interaction, extracellular matrix homeostasis, drug detoxification, immune tolerance, etc., and may secrete cytokines (including hepatocyte growth factors) to maintain the quality of liver cells. The activation of HSCs—conversion of quiescent vitamin A storage cells into myofibroblasts [6] which are proliferative, contractile, inflammatory

and chemotactic has been regarded as the main driver of liver fibrosis [7], thus ECM production is enhanced [8]. Paracrine signals from damaged epithelial cells, fibrotic tissue microenvironment, disorders of immunity and systemic metabolism, intestinal dysbiosis and hepatitis virus products can directly or indirectly induce HSCs activation [9]. Activated HSCs further stimulate the activation of surrounding HSCs and other cellular biological behaviors in an autocrine and paracrine manner [10]. Liver fibrosis is a dynamic and reversible process, and several clinical studies have also shown the regression of fibrosis and the reversal of cirrhosis in biopsy specimens. Therefore, it is of great significance to clarify the mechanism of liver fibrosis [11].

Septins are a family of evolutionarily highly conserved GTP-binding proteins which are ubiquitously expressed in all eukaryotes except higher plants, and this family contains 13 members [12]. They are likely to be a new class of cytoskeletal components [13] that have been shown to act as a scaffolding protein and regulate a range of cellular functions such as cell division, apoptosis, migration, polarization, endocytosis and so on via recruiting and blocking related proteins [14]. The complex signaling pathways of various gene proteins between the septins family make more and more people believe that septins may become a new target in anti-cancer treatment strategies [15]. Therefore, it is of great significance to focus on the relationship between the molecular and cellular pathophysiological mechanisms of septin's influence in cancer and other diseases.

Among the 13 members of the septin family, SEPT6 is expressed in all tissues, especially in neuron tissue [16] and lymphoid [17]. Studies have shown that SEPT6 forms a heteromeric complex with SEPT2 and SEPT7 (SEPT2/6/7), which is related to actin stress fibers and can regulate the microtubule cytoskeleton during mitosis. Therefore, this complex may regulate cell cycle and proliferation [18]. Inhibition of SEPT2, 6 and 7 leads to the breakdown of stress fibers and through the inhibition of cytokine signaling 7/NCK adaptor protein 1 (SOCS7/NCK) signaling inhibitors, the cells lose their polarity, causing DNA damage and cell cycle stagnation [19]. Previous research demonstrated SEPT6 is down-regulated in prostate cancer, and low expression of SEPT6 could promote cell survival, migration and invasion, which indicated SEPT6 to be a tumor suppressor in prostate cancer [20]. However, another study demonstrated that SEPT6 is up-regulated by HBsAg in HepG2.2.15 and promotes HCC proliferation [21], which suggested SEPT6 plays a tissue-specific role.

SEPT6 mainly regulates actin dynamics, cell shape and microtubule dynamics, cytokinesis, cell proliferation, cell cycle progression, cell apoptosis and cell migration. But the role of SEPT6 and its mechanism in liver fibrosis has not been studied before. Therefore, in the manuscript entitled "Effect of SEPT6 on the biological behavior of hepatic stellate cells and liver fibrosis in rats and its mechanism", we explored the role of SEPT6 in liver fibrosis and elucidated the underlying mechanism [22].

We first detected the level of the whole septin family member in healthy and fibrotic rat liver and found SEPT6 is elevated significantly. The rat hepatic fibrosis model was established by thioacetamide (TAA). By performing real-time PCR, western blot, immunohistochemistry and immunofluorescence, we found SEPT6 is expressed both in HSCs and hepatocytes, and its expression is upregulated in liver cirrhotic patients and TAA-induced hepatic fibrosis rats. We further explored the effects of SEPT6 in biology behavior of HSCs by using gain- and loss- of function assays both *in vitro* and *in vivo*. We found inhibition of SEPT6 by siRNA can down-regulate the expression of α -SMA and COL1A1 in HSCs, inhibit the proliferation of HSCs, promote G1/S cell cycle arrest and decrease the expression of cyclin D1 in HSCs, promote early apoptosis of HSCs, increase the expression of BAX and decrease the expression of BCL-2, and decrease the expression of migration related genes such as MMP2 and MMP9. Conversely, overexpression of SEPT6 exerts the opposite effect mentioned above. What's more, inhibition of SEPT6 expression could downregulate the protein level of TGF- β 1 and the phosphorylation of Smad2, Smad3 in TGF- β 1 signaling pathway, ERK, JNK, P38 in MAPK signaling pathway and AKT in PI3K/AKT

signaling pathway, indicating that SEPT6 could regulate the biological behavior of HSCs through the signaling pathways mentioned above. In animal experiments, we used TAA to establish rat hepatic fibrosis model. By performing Hematoxylin and eosin (HE), Masson's trichrome, Sirius Red staining, Real-time PCR, western blot, Ki67 and TUNEL staining, we found adenovirus mediated SEPT6 inhibition could block the progression of established fibrosis despite continued liver injury following *in vivo* administration.

Liver fibrosis is a necessary process from various chronic hepatitis to liver cirrhosis or pathological development of liver cancer, the regeneration of liver cells caused by inflammatory injury mostly comes from the activation of HSCs. A variety of biological changes have occurred in the activated HSCs, such as promoting fibrous tissue proliferation, migration and secreting a large number of cytokines which have anti-apoptotic effect and could promote cell proliferation. The abnormal growth of hepatocyte, disordered gene expression and DNA damage caused by chronic inflammation further lead to the malignant development of liver tissue and eventually induce HCC. It can be seen that the occurrence and development of HCC is closely related to the potential cancer microenvironment generated by hepatitis and liver fibrosis.

HCC is a heterogeneous disease with rising incidence and mortality rate, one of the most common malignancies and the third leading cause of cancer-related mortality around the world [23]. Although surgical resection and liver transplantation are curative therapies for HCC patients, the incidence rate of HCC approximates the mortality rate because most HCC patients are diagnosed at advanced stage, only less than 40% of the patients are diagnosed at the early stage [24]. The overall prognosis remains dismal, mainly attributed to recurrence and metastasis [25]. Therefore, novel causative genes and molecular mechanism underlying HCC progression and metastasis needs to be identified to develop better therapeutic targets.

As we discussed above, SEPT6 was up-regulated in liver fibrosis and promoted hepatic stellate cells activation, proliferation and migration. As liver fibrosis and the subsequent cirrhosis are considered to be precancerous states of HCC, it is logically reasonable that SEPT6 might promote HCC progression. What's more, accumulating studies have shown that cytoskeleton proteins play important roles in the tumorigenesis of hepatocellular carcinoma. However, the role of SEPT6 in hepatocellular carcinoma remains unknown. Thus, we want to continue the previous research and explore the role and mechanism of SEPT6 in HCC.

In the new study, we detected SEPT6 in HCC cell lines using RT-PCR and Western blot assay, and performed gain- and loss- of function assays to detect the role of SEPT6 in the proliferation, cell cycle progression, migration and invasion of HCC. Then, we explored the underlying mechanism focused on the Hippo signaling as the pathway plays vital role in HCC tumorigenesis and progression. We found that SEPT6 is significantly up-regulated in HCC tissue and highly metastatic potential HCC cell lines, and demonstrated that SEPT6 acts as an oncogene in HCC progression. We proved that SEPT6 overexpression inactivated the Hippo signaling, dephosphorylated and stabilized the downstream effector YAP, subsequently, active YAP translocated into the nucleus, where it promoted transcription of the target genes. The new project is still in progress, all the data we have now indicates an oncogenic role of SEPT6, which may play a role through a novel SEPT6/Hippo/YAP axis, thus providing a potential therapeutic insight into HCC therapy.

TGF- β 1, which was mentioned in this manuscript, has been considered as one of the most important cytokines and plays a vital role in regulating the invasion and metastasis of the advanced tumors. Two classic TGF- β 1 signaling pathway is eliciting increasing attention in cancer therapy. One is TGF β -T β R-Smads pathway, and another is TGF β -T β R-TAB1/TAK1-MKK3-p38. TGF- β can also promote tumor development and metastasis by affecting the tumor microenvironment. All these indicate that TGF- β is a potential tumor therapy target, and actually several therapeutic methods have been tried to target the TGF- β 1 signaling pathway recently. Thus, we want to further explore the relationship between SEPT6 and TGF- β 1 in order to seek inhibitors to treat HCC. Besides, it is also worthy to investigate the effect of SEPT6 in HCC by establishing more clinically practical and meaningful animal models. In this way, we might develop effective therapeutic target to control distant metastasis of HCC.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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