

Viral protein revives host cell miRNA function by dampening the circular RNA sponge

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In this study, Liu *et al.* have investigated the inhibitory effects of hepatitis B viral X gene-coded protein (HBx) on circular RNA circSFMBT2 expression in host cells in connection to hepatocellular carcinoma (HCC) cell metastasis. The authors have delineated a novel mechanism to show how HBx lowers circSFMBT2 expression to upregulate miR-665 activity linked with miR-665 target gene TIMP3 downregulation and enhanced HCC metastasis.¹ They also found that circSFMBT2 RNA acts as sponge for miR-665 in hepatic cells.

Hepatic cancer is a serious health issue globally, and the majority of liver cancer is caused by hepatitis B virus.² The effect of viral proteins on hepatocyte growth and proliferation has been extensively investigated recently and those studies have revealed altered cell signaling and metabolic pathways caused by viral factors in host cells. Mechanistic understanding of how virus-derived factors disturb host cells' machineries is essential to understand the HCC development process and will be the key for any new therapy development for hepatitis B.

microRNAs (miRNAs) are important post-transcriptional gene regulators that, by base pairing to target mRNAs, regulate the majority of metabolic and other regulatory pathways in higher eukaryotes. It has been recently being shown that other non-coding RNAs, like competing endogenous RNA (ceRNA), and circular RNAs can affect the miRNA function by base pairing with them.³ The circular RNAs are generated through a back-splicing reaction, and their biogenesis and turnover processes are highly regulated in higher metazoans. Therefore, dampening the regulatory circular RNA level will be an effective way of controlling the miRNA function.

In their study, Liu *et al.* have identified a circular RNA, namely circSFMBT2, as a key player in mediating the pro-metastatic effects of HBx, the viral effector protein. Interestingly, low expression of circSFMBT2 was found to be associated with poor prognosis and vascular invasion of patients with HCC.¹ The authors went on to find the mechanism of downregulation of circSFMBT2 by HBx and have shown how HBx, by interacting with DEXH-box helicase 9 (DHX9) protein of the host cell, downregulates circSFMBT2 formation in hepatic cells. The authors also have identified circSFMBT2 as the regulator of miR-665. In the presence of HBx, the downregulation of circSFMBT2 was found to be associated with an increase in miR-665 activity that, in turn, lowers the expression of its target TIMP3, the known suppressor of tumor cell metastasis. The authors have used RNA sequencing (RNA-seq) experiments done with cells expressing or not expressing circSFMBT2 to identify the differentially expressed genes, and among these, TIMP3 was identified as one of the genes that is controlled by miR-665, the miRNA having potential binding sites also on circSFMBT2 RNA (Figure 1). In subsequent experiments, the authors confirmed the sponging effect of circSFMBT2 for miR-665 in hepatic cells.

Viral proteins usually have diversity in their function, and they must have co-evolved with their host to adopt the regulatory function in the host cells. Interestingly, HBx is known to act as oncogene in both genetic regulation and epigenetic modification observed in HCC. The presence of possible intrinsically disordered structures in HBx protein may be the structural basis for the promiscuous structure-function behavior of this protein. HBx also has pleomorphic effects on host cell proliferation pathways

while interfering with DNA-repair mechanisms. HBx is known to function as non-coding RNA regulator.⁴ In this study, Liu *et al.* have revealed the direct interaction of HBx with DHX9. Knockout experiments, targeted to remove the flanking *Alu* sequence, revealed a possible interaction of HBx/DHX9 with *Alu* elements to control back splicing of the pre-SFMBT2 and a retarded generation of the mature circular form. The conclusive data for direct binding of DHX9 with circSFMBT2 is missing. However, circumferential evidence strongly suggests a negative role of DHX9 on biogenesis of circSFMBT2 only in presence of HBx.

The other potential caveat could have been the mode of action of circSFMBT2 on miRNAs. Specific influence of circSFMBT2 on miR-665 has been studied in this report.¹ The possible base pairing between the circSFMBT2 and miR-665 was found to be necessary to cause a "sponging effect" to remove the functional pool of miR-665 by circSFMBT2 that allows the expression of miR-665 targets in cells expressing circSFMBT2. This work of Liu *et al.* highlighted the potential of circular RNAs to be used as a target to combat HCC cell metastasis where the processing step of the circular RNA or its interaction with cognate miRNAs may also be targeted to control HCC metastasis and proliferation steps. It will be highly interesting to know whether such a regulation is prevalent with other virus-encoded proteins, which, also by targeting expression of circular RNA sponges, alter the miRNA expression, abundance, and turnover in disease cells and contribute to infection niche establishment process. Specific protein factors that, either by promoting the miR-665 and circSFMBT2 interaction or controlling the stability and compartmentalization of the circular RNA-miRNA complex, can ensure additional levels of regulation

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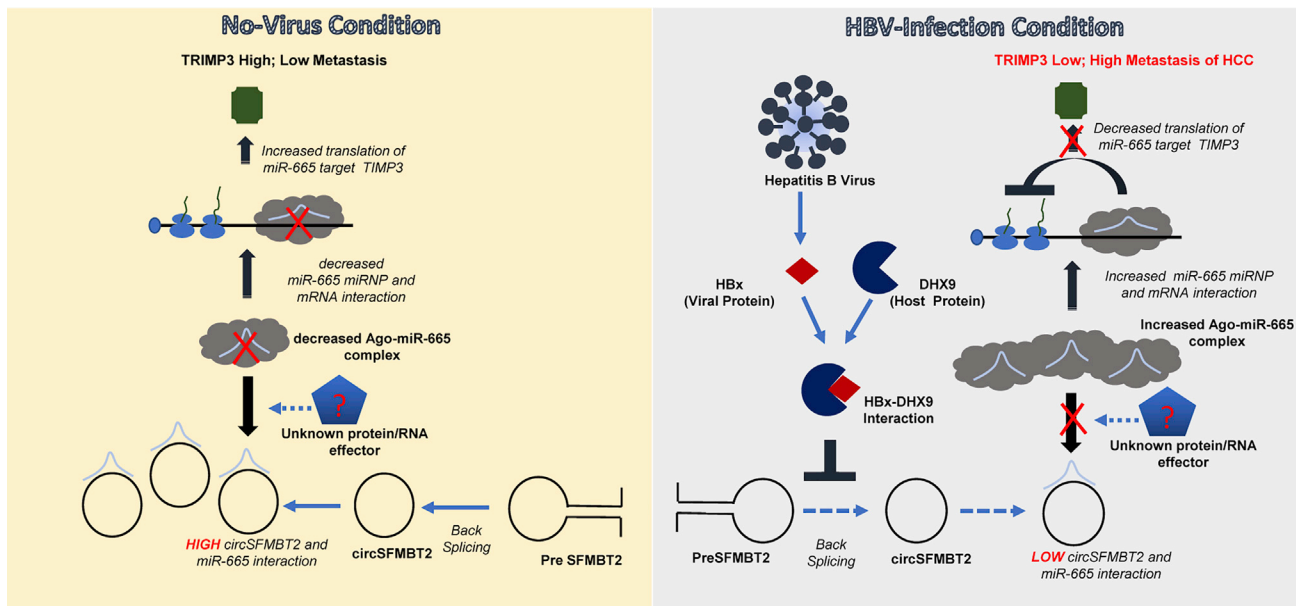


Figure 1. Viral protein HBx restricts expression of circular RNA circSFMBT2 to upregulate miRNA-665 and induces metastasis of HCC cells

The study by Liu *et al.* has shown how the hepatitis B virus-derived protein HBx by interacting host cell factor DHX9 influences the biogenesis of circSFMBT2 from its precursor in infected hepatocytes. The decreased level of circSFMBT2 fails to sponge miR-665, and the increased level of miR-665 miRNPs is associated with reduced expression of miR-665 target genes including TIMP3, a known inhibitor of cell metastasis process, in hepatocellular carcinoma cells. The presence of an additional protein or RNA factor that can potentially influence the miR-665 and circSFMBT2 interaction or the stability of the miRNA-circular RNA complex can attribute to the effective miR-665 sponging effect exerted by circSFMBT2.

(Figure 1). The stoichiometry of the “sponge” circular RNA and the target miRNA is also an important determinant to have viable inhibitory effect of the sponge RNA on cognate miRNA. This should also be dependent on the binding strength of miRNA-circular RNA pairs. Therefore, information on the presence of additional regulatory pathways that are favoring or countering the interaction step of miRNA and circular RNA sponge is important to understand the biology of viral factor-driven changes in miRNA expression via circular RNAs.

AUTHORS CONTRIBUTIONS

S.N.B. has conceived and written this commentary.

DECLARATION OF INTERESTS

The author has no conflict of interest to declare.

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