

Can tobacco use promote HCV-induced miR-122 hijacking and hepatocarcinogenesis?

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Lijun Zhao, Farong Li, Ethan Will Taylor. Can tobacco use promote HCV-induced miR-122 hijacking and hepatocarcinogenesis? *Medical Hypotheses*. Volume 80, Issue 2, February 2013, Pages 131-133. <https://doi.org/10.1016/j.mehy.2012.11.009>



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Abstract:

Chronic hepatitis C virus (HCV) infection is a well-recognized risk factor for hepatocellular carcinoma (HCC). As a co-risk factor, the role of tobacco use in HCV-driven carcinogenesis and relevant underlying mechanisms remain largely unclear. The latest discoveries about HCV replication have shown that HCV RNA hijacks cellular miRNA-122 by forming an Ago2-HCV-miR-122 complex that stabilizes the HCV genome and enhances HCV replication. Our previous work has demonstrated that aqueous tobacco smoke extract (TSE) is a potent activator of HIV replication via TSE-mediated viral protection from oxidative stress and activation of a set of genes that can promote viral replication. Since HCV is, like HIV, an enveloped virus that should be equally susceptible to lipid peroxidation, and since one of the TSE-upregulated genes, the DDX3 helicase, is known to facilitate HCV replication, we hypothesize that (1) tobacco use can similarly enhance HCV viability and replication, and promote HCC progression by up-regulation of DDX3, and (2) by competing for binding with miR-122 as a competing endogenous RNA (ceRNA), HCV replication can liberate miR-122's direct target, oncogenic gene cyclin G1 (CCNG1); furthermore, simultaneous tobacco use can synergistically enhance this competing effect via HCV upregulation. Our hypotheses may lay a foundation for better understanding of carcinogenesis in HCV-driven HCC and the potential role of tobacco as a cofactor. Disrupting the HCV ceRNA effect may provide a new strategy for designing anti HCV/HCC drugs.

Keywords: hepatitis C virus | HCV | hepatocarcinogenesis | tobacco use

Article:

Introduction

Chronic hepatitis C virus (HCV) infection is a well recognized risk factor for hepatocellular carcinoma (HCC) [1]. Tobacco use increases the risk of HCC in those with HCV infection [2], [3]. However, the molecular mechanisms that underlie the interaction between tobacco use and HCV infection and the consequent carcinogenesis remain largely unclear.

Elucidation of the underlying mechanisms may not only reveal the role of tobacco use in HCV-driven HCC from a public health perspective, but also provide new targets for developing anti-HCV/HCC drugs.

A body of evidence suggests that liver specific microRNA miR-122 promotes HCV replication by binding with 5' terminal nucleotides of the HCV genome [4], with the Ago2-miR-122 complex masking and stabilizing HCV RNA against 5' exonuclease degradation [5], [6], [7]. The interaction between miR-122 and HCV RNA implies that HCV replication can sequester miR-122, as suggested by Lemon and coworkers [7]. A novel mechanism for regulation of gene expression has been suggested recently, known as competing endogenous mRNA (ceRNA) [8]. Mechanistically, two mRNAs sharing common microRNAs will compete for binding to those microRNAs. Thus, the transcription of one gene will compete for binding to the shared microRNA and liberate the RNA of the other gene, increasing its expression. This mechanism for the regulation of gene expression has been proven in several cases [9], [10].

Human DDX3, a DEAD (Asp-Glu-Ala-Asp)-box RNA helicase, is involved in HCV translation, replication and hepatocarcinogenesis [11], [12]. Up-regulation of DDX3 promotes the expression of Snail, an inducer of the epithelial to mesenchymal transition (EMT), thereby contributing to the progression of some cancers [12], such as in the transformation of breast epithelial cells [13].

Our previous study [14] revealed that tobacco smoke extract (TSE) can enhance HIV viability, replication and up-regulate DDX3 in T cells.

The hypotheses

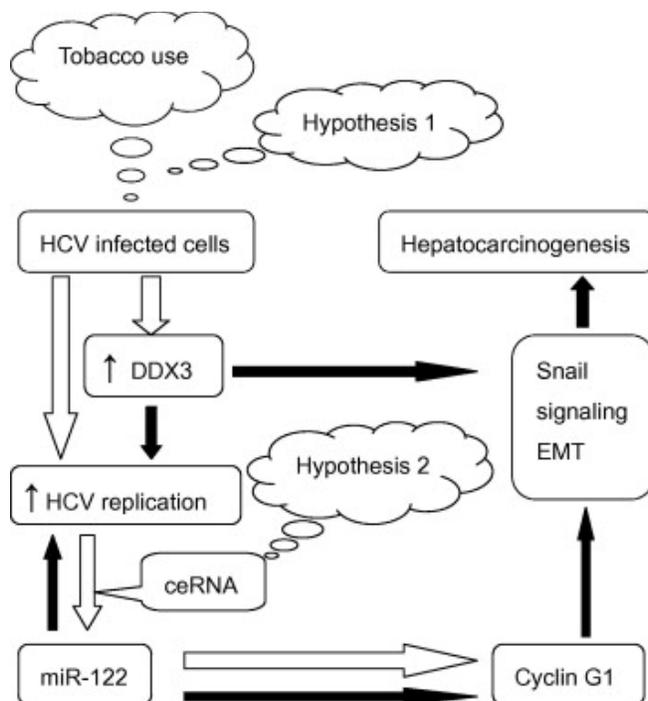


Figure 1. Hypothesis chart. (→)Known flow (⇌) Hypothesized flow EMT: epithelial-mesenchymal transition.

Based on this background and the results of our previous study, we hypothesize that tobacco use can promote HCV-driven hepatocarcinogenesis. Mechanistically, tobacco use can enhance HCV viability and replication, and promote HCC progression, either via its anti-oxidant potential or by up-regulating DDX3, and HCV replication can act as ceRNA to liberate miR-122's direct target, oncogenic gene CCNG1; simultaneous tobacco use can synergistically enhance this ceRNA effect via HCV upregulation (Fig. 1).

Evaluation of the hypotheses

The direct effect of tobacco use on HCV replication

The current knowledge that tobacco use has a synergistic effect on HCV-driven HCC is mainly from epidemiological studies [1], [2], [15], [16]. The underlying molecular mechanisms for the direct interactions between tobacco use and HCV infection have not been definitively established. So far, few studies have been performed at the cellular and genetic level to elucidate their interactions in hepatocarcinogenesis. In our previous study [14], we showed that TSE has anti-oxidant potential to protect HIV and HIV-infected cells from peroxidative stress; TSE can also induce the up-regulation of DDX3 in human T cells. Since TSE used in the experiment has a nicotine concentration within the range of that in the plasma of smokers, we therefore assume the effect of TSE in our in vitro experiments is equivalent to that in vivo. Because HCV, like HIV, is an enveloped virus susceptible to lipid peroxidation, and thus could benefit from the protective effect of TSE, and since DDX3 is important for HCV replication, we hypothesize that tobacco smoke has similar positive effects on HCV replication. Given that DDX3 is required for HCV replication [11], [17], [18], our hypothesis will bridge the gap between the role of tobacco use and HCV replication, via the role of DDX3.

Genes and transduction signaling pathways involved in the effect of tobacco use on HCV-driven carcinogenesis

Epidemiological studies have shown tobacco use is a co-risk factor for HCV-driven HCC, but the molecular link between tobacco use and HCC has not been fully defined. We have identified a direct effect of TSE on the up-regulation of DDX3 [14]. Significantly, DDX3 has ability to promote cancer progression by up-regulating the expression of Snail, a transcription factor known as an inducer of EMT [12]. Our hypothesis of the direct effect of TSE on the expression of DDX3 and the consequent activation of EMT signaling may provide a molecular link between tobacco use and hepatocarcinogenesis. The role of DDX3 in HCV replication has been well established by several studies [11], [18], [19]. Again, our published study shows that TSE has the ability to stimulate the up-regulation of DDX3 in T cells. Since HCV infection and tobacco use are co-risk factors in the progression of HCC, there is a high probability that tobacco use could enhance HCV replication through activation of DDX3. Establishment of a probable role of DDX3 in tobacco-induced progression of HCV infection may not only reveal the mechanism of hazards of smoking, but also implicate DDX3 as a therapeutic drug target for HCV infection.

ceRNA theory describes the “sequestering” effect of HCV on liver specific miR-122 and its consequences

It is quite clear that liver specific miR-122 has a unique ability to bind to HCV genomic RNA to stimulate HCV replication [6], [7], [20]. In turn, HCV replication will “sponge up” miR-122, as Lemon and coworkers have proposed, via a “hijacking” or “sequestering” effect [7]. We invoke a recently discovered gene expression regulation mechanism, ceRNA theory [8], [10], to describe the proposed “hijacking” effect and extend to its consequences. In this case, HCV RNA and CCNG1, the direct target gene of miR-122, will compete for binding of limited amounts of cellular miR-122. The more HCV RNA, the more miR-122 will be sequestered, and the more CCNG1 gene expression will be rescued. miR-122 has liver-specific functions in the regulation of lipid metabolism, inflammation and regulation of its direct target gene, CCNG1 [21]. With “hijacking”, HCV replication and the enhancing effect of tobacco use on HCV will greatly liberate the direct target of miR-122 and result in the rescuing of CCNG1, an oncogenic gene that has been identified as a major player in carcinogenesis in liver [22] and other cancers [23]. The main downstream signaling of CCNG1 is the process of EMT [22], which can converge with the effect of DDX3 on Snail signaling as stated above. Based on these factors, we hypothesize a mechanism whereby the HCV ceRNA effect is expected to promote hepatocarcinogenesis, and tobacco use will enhance the effect. Assessment of this HCV ceRNA hypothesis may advance our understanding of the interactions between HCV infection and host cells and their roles in hepatocarcinogenesis, and may link HCV replication and CCNG1 oncogenic signaling.

Discussion

Our hypotheses focus on direct interactions between tobacco smoke and virus activation, and invoke a HCV “ceRNA theory” as a molecular mechanism for HCV-driven carcinogenesis.

Given the dual functions of miR-122 in HCV infection [20], [24], [25] and HCC carcinogenesis [21], several therapies based on miR-122 have been suggested [21], [26], [27]. On one hand, being an enhancer of HCV replication, miR-122 is a target for silencing in the treatment of HCV [26], [28]. However, deletion of miR-122 in mouse liver results in hepatosteatosis, hepatitis, and the development of tumors resembling HCC [21]. On the other hand, miR-122 inhibits tumorigenesis by directly targeting oncogenic CCNG1, as well as Wnt/ β -catenin and N-myc downstream-regulated gene 3 (NDRG3) pathways [25]. In the EMT of HCC, the down-regulation of miR-122 [29] gives rise to up-regulation of CCNG1 and consequent Snail and PI3 K/Akt activation [22]. In patients with hepatitis B, loss of miR-122 enhances HBV replication via CCNG1-modulated p53 activity [30]. As a tumor-suppressor, delivery of miR-122 to a MYC-driven mouse model of HCC has shown a strong inhibitory effect on tumorigenesis [21]. Nevertheless, a potential side effect of miR-122 mimicry therapy for HCC cannot be excluded due to its enhancing effect on HCV replication. Because of the dual roles of miR-122 in both HCV infection and hepatocarcinogenesis, a balanced miR-122 based therapy for HCV/HCC is required [27].

Since up-regulation of DDX3 induced by TSE and up-regulation of CCNG1 induced by the HCV ceRNA effect both have a role in promoting EMT through Snail and PI3 K/Akt signaling [12], [22], elucidation of the HCV ceRNA theory will lay a foundation for better understanding of carcinogenesis in HCV-driven HCC and the potential role of tobacco as a cofactor. Disrupting the HCV ceRNA effect, not merely silencing or over-expressing miR-122,

will provide a new strategy for designing anti HCV/HCC drugs. Our hypotheses may also have an impact on public awareness about the hazards of tobacco use, especially for patients with viral hepatitis.

Conflict of interest statement

None declared.

Acknowledgment

This work was supported by a Grant from China National Natural Science Foundation to Dr. Lijun Zhao (No. 81272257).

References

- [1] Sherman M, Llovet JM. Smoking, hepatitis B virus infection, and development of hepatocellular carcinoma. *J Natl Cancer Inst* 2011;103:1642–3.
- [2] Chuang SC, Lee YC, Hashibe M, Dai M, Zheng T, Boffetta P. Interaction between cigarette smoking and hepatitis B and C virus infection on the risk of liver cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2010;19:1261–8.
- [3] Kuper H, Tzonou A, Kaklamani E, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer* 2000;85:498–502.
- [4] Jangra RK, Yi M, Lemon SM. Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122. *J Virol* 2010;84:6615–25.
- [5] Jopling CL, Norman KL, Sarnow P. Positive and negative modulation of viral and cellular mRNAs by liver-specific microRNA miR-122. *Cold Spring Harb Symp Quant Biol* 2006;71:369–76.
- [6] Machlin ES, Sarnow P, Sagan SM. Masking the 50 terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. *Proc Natl Acad Sci USA* 2011;108:3193–8.
- [7] Shimakami T, Yamane D, Jangra RK, et al. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. *Proc Natl Acad Sci USA* 2012;109:941–6.
- [8] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 2011;146:353–8.
- [9] Cesana M, Cacchiarelli D, Legnini I, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 2011;147:358–69.
- [10] Tay Y, Kats L, Salmena L, et al. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. *Cell* 2011;147:344–57.

- [11] Ariumi Y, Kuroki M, Abe K, et al. DDX3 DEAD-box RNA helicase is required for hepatitis C virus RNA replication. *J Virol* 2007;81:13922–6.
- [12] Sun M, Song L, Zhou T, Gillespie GY, Jope RS. The role of DDX3 in regulating Snail. *Biochim Biophys Acta* 1813;2011:438–47.
- [13] Botlagunta M, Vesuna F, Mironchik Y, et al. Oncogenic role of DDX3 in breast cancer biogenesis. *Oncogene* 2008;27:3912–22.
- [14] Zhao L, Li F, Zhang Y, et al. Mechanisms and genes involved in enhancement of HIV infectivity by tobacco smoke. *Toxicology* 2010;278:242–8.
- [15] Hassan MM, Spitz MR, Thomas MB, et al. Effect of different types of smoking and synergism with hepatitis C virus on risk of hepatocellular carcinoma in American men and women: case-control study. *Int J Cancer* 2008;123:1883–91.
- [16] Fujita Y, Shibata A, Ogimoto I, et al. The effect of interaction between hepatitis C virus and cigarette smoking on the risk of hepatocellular carcinoma. *Br J Cancer* 2006;94:737–9.
- [17] Garbelli A, Radi M, Falchi F, et al. Targeting the human DEAD-box polypeptide 3 (DDX3) RNA helicase as a novel strategy to inhibit viral replication. *Curr Med Chem* 2011;18:3015–27.
- [18] Angus AG, Dalrymple D, Boulant S, et al. Requirement of cellular DDX3 for hepatitis C virus replication is unrelated to its interaction with the viral core protein. *J Gen Virol* 2010;91:122–32.
- [19] Owsianka AM, Patel AH. Hepatitis C virus core protein interacts with a human DEAD box protein DDX3. *Virology* 1999;257:330–40.
- [20] Henke JI, Goergen D, Zheng J, et al. MicroRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J* 2008;27:3300–10.
- [21] Hsu SH, Wang B, Kota J, et al. Essential metabolic, anti-inflammatory, and antitumorigenic functions of miR-122 in liver. *J Clin Invest* 2012;122:2871–83.
- [22] Wen W, Ding J, Sun W, et al. Cyclin G1-mediated epithelial-mesenchymal transition via phosphoinositide 3-kinase/Akt signaling facilitates liver cancer progression. *Hepatology* 2012;55:1787–98.
- [23] Ye XX, Liu CB, Chen JY, Tao BH, Zhi-Yi C. The expression of cyclin G in nasopharyngeal carcinoma and its significance. *Clin Exp Med* 2012;12:21–4.
- [24] Jopling CL. Regulation of hepatitis C virus by microRNA-122. *Biochem Soc Trans* 2008;36:1220–3.

[25] Sendi H. Dual role of miR-122 in molecular pathogenesis of viral hepatitis. *Hepat Mon* 2012;12:312–4.

[26] Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010;327:198–201.

[27] Young DD, Connelly CM, Grohmann C, Deiters A. Small molecule modifiers of microRNA miR-122 function for the treatment of hepatitis C virus infection and hepatocellular carcinoma. *J Am Chem Soc* 2010;132:7976–81.

[28] Branch AD, Rice CM. Antisense gets a grip on miR-122 in chimpanzees. *Sci Transl Med* 2010;2:13ps11.

[29] Gramantieri L, Ferracin M, Fornari F, et al. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007;67:6092–9.

[30] Wang S, Qiu L, Yan X, et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1)-modulated P53 activity. *Hepatology* 2012;55:730–41.