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Cellular microRNA and the tumorigenesis of hepatocellular carcinoma

Yu Li,* Kris V. Kowdley*

* Benaroya Research Institute and Center for Liver Disease, Digestive Disease Institute Virginia, Mason Medical Center, Seattle Washington, USA.

Article commented

Jin-fang Zhang, Ming-liang He, Wei-ming Fu, Hua Wang, Lian-zhou Chen, Xiao Zhu, Ying Chen, Dan Xie, Paul Lai, Gong Chen, Gang Lu, Marie C.M. Lin, and Hsiang-fu Kung. Primate-specific microRNA-637 inhibits tumorigenesis in hepatocellular carcinoma by disrupting signal transducer and activator of transcription 3 signaling. *Hepatology* 2011; 54: 2137-48.

Comments

Hepatocellular carcinoma (HCC) is one of the most malignant solid tumors causing over 650,000 global deaths each year.[1] Attributed to complex mechanisms, the tumorigenesis of HCC is associated with a broad range of risk factors, including HBV/HCV infection, heavy alcohol consumption, and non-alcoholic steatohepatitis (NASH).[2] Although the underlying mechanisms of the HCC tumorigenesis is not fully understood, it should be noted that the onset of HCC is often accompanied by inflammation and aberrant proliferation of hepatocytes stimulated by pro-inflammatory cytokines in the liver.[3,4] Hence, liver inflammation and deregulated hepatocyte proliferation have been postulated as major contributing factors to the tumorigenesis of HCC. Interestingly, the signal transducer and activator of transcription 3 (STAT3) may play critical roles in HCC tumorigenesis due to its involvement in both processes.[5,6] The phosphorylation of STAT3, induced by extracellular stimuli such as pro-inflammatory cytokines, results in its subsequent translocation into the nucleus; once in the nucleus, STAT3 regulates the transcription of many genes, some important to tumorigenesis.[7] Notably, STAT3 activation is prevalent in malignant tissues in HCC tumors from patients with poor prognosis, but not in adjacent normal tissues.[8,9] STAT3 activation is transient in normal cells but is constitutive in cancer cells, suggesting its close association with tumorigenesis. However, it remains unclear how the constitutive activation of STAT3 is maintained throughout the tumorigenesis of HCC.

As negative regulators of gene expression, microRNAs play key roles in a variety of critical biological processes, many of which are associated with the progression and prognosis as well as response to treatment of many tumors.[10] Interestingly, recent studies have indicated that the expression of tumor-associated microRNAs can be directly induced by STAT3.[11-13] Nevertheless, the relationship between STAT3 and microRNAs seems more complicated. Zhang, *et al.* demonstrated that a primate-specific microRNA-637 was able to negatively regulate the STAT3 activity by repressing the expression of the pro-inflammatory cytokine leukemia inhibitory factor (LIF). In this study, the authors not only provided evidence from both *in vitro* and *in vivo* experiments elucidating the regulatory relationship between miR-637 and LIF, but also demonstrated significant growth retardation in tumors caused by over-expression of miR-637 in a murine model.

In this fine work, the authors initially demonstrated that miR-637 expression level was significantly lower in HCC cell lines and tumor specimens compared with that in non-HCC tissues. They also showed the inverse relationship between the levels of miR-637 expression and FBS-induced cell proliferation. More importantly, miR-637 over-expression in HCC cells strongly repressed cell proliferation and simultaneously induced apoptosis, which suggested an anti-tumor effect of miR-637. To elucidate the role of miR-637 in HCC tumorigenesis, the authors identified potential targets of miR-637 related

Correspondence and reprint request: Kris V. Kowdley, M.D.
Benaroya Research Institute and Digestive Disease Institute
Virginia Mason Medical Center
1201 Ninth Ave, Seattle, WA 98101
Ph.: 206-223-2319. Fax: 206-341-1188
E mail: Kris.Kowdley@vmmc.org

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to HCC formation using bioinformatic approaches. They found that both STAT3 and its activation inducer LIF were predicted targets of miR-637. To validate the regulatory relationship between miR-637 and these two targets, a reporter system was used to confirm the miR-637 mediated repression on LIF and STAT3 expressions. It turned out that LIF, but not STAT3, was a direct target of miR-637. The repression of LIF by miR-637 resulted in down-regulation of STAT3 phosphorylation and therefore its activity. More importantly, STAT3 phosphorylation could be restored by supplementing exogenous LIF in the culture, suggesting that miR-637 was able to regulate STAT3 activity by controlling the level of LIF expression. In addition, the authors used a murine model to investigate the role of miR-637 in the context of HCC. In nude mice injected with hepatoma cells that over-expressed miR-637, the tumors remained significantly smaller than the tumors in the controls. A closer look at the levels of activated STAT3 in these tumors clearly revealed that increased miR-637 expression correlated with decreased STAT3 phosphorylation. Although the authors did not provide data indicating an inverse relationship between LIF and miR-637 in mice, they demonstrated this relationship in human HCC samples. Taken together, the evidence from this study strongly points to a protective role of miR-637 against HCC tumorigenesis and development.

The work of Zhang, *et al.* is particularly interesting because they provided a mechanistic model potentially useful for developing siRNA-based HCC therapeutics. As most anti-cancer drugs with the exception of sorafenib are largely ineffective in treating HCC, siRNA-based therapeutics may be a viable alternative as they are effective, safe, easy to design and amenable to commercial production. Furthermore, a number of siRNA drugs have demonstrated their efficacy in treating advance cancer,[14] viral diseases,[15,16] and hypercholesterolemia with more clinical trials planned.

Several caveats must be considered in interpreting the results of Zhang, *et al.,* to address the toxicity and safety of miR-637 as an anti-tumor drug candidate. First, the effect of miR-637 over-expression in hepatocytes needs to be assessed in a broader scope. Since a microRNA is able to repress the expression of dozens or even hundreds of cellular genes, other pathways associated with the predicted miR-637 targets could be analyzed by bioinformatics to identify potential adverse effects. In addition, high-throughput genomics and next-generation sequencing technologies could be used to detect non-specific effects of miR-637 over-expression that cannot be identified by bioinformatics alone. Second, it would be useful to identify other cellular microRNAs that also repress the LIF expression for designing siRNA drugs with fewer "off-target" effects. Combination therapy using multiple microRNAs targeting the same gene would likely require a lower effective dose, relative to the use of just a single microRNA at a high dosage, but with minimized off-target effects.

In conclusion these exciting findings offer new promise in understanding the pathogenesis of HCC and may open new avenues to novel therapeutics against this important liver disease.

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