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MiRNAs control hepatocarcinogenesis by regulating hepatocyte nuclear factor 4 α -inflammatory signal feedback loops

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Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the third leading cause of cancer mortality, with a prevalence of approximately 600,000 cases worldwide.¹ Over the last 20 years, HCC incidence has risen steadily in both western countries, such as the United States, and developing countries, including China. HCC is commonly associated with chronic hepatitis B and C virus infections, alcohol abuse, and obesity. In spite of the etiologies of this disease, most HCCs are associated with predisposing liver disease with unresolved inflammation.² This chronic inflammation status alters the hepatic microenvironment, favoring pro-oncogenic or (epi)genetic changes, driving hepatocarcinogenesis. Over the last twenty years, many studies have suggested that chronic liver inflammation is tightly controlled by the interaction of a variety of inflammatory mediators and their downstream signaling pathways. In this context, both NF- κ B and STAT3 have been identified as two key signaling molecules that link inflammation and liver cancer.^{3, 4}

In contrast to the constitutive activation of chronic inflammatory signals that usually accelerate hepatocarcinogenesis, the expression of hepatocyte nuclear factor 4 α (HNF4 α), a master regulator of hepatocyte differentiation, is suppressed in liver cancer. HNF4 α is primarily expressed in hepatocytes and indispensable for hepatic epithelium formation during embryonic development. HNF4 α is widely associated with the transcriptional regulation of hepatocyte genes specifically implicated in lipid metabolism, glucose metabolism, differentiation, and morphogenesis.⁵ Apart from its function in hepatocyte differentiation, HNF4 α can also inhibit hepatocyte proliferation and hepatocarcinogenesis by suppressing various pro-mitogenic genes.^{6–8} Loss of HNF4 α promotes chemically induced liver cancer in rodents and is associated with human HCC.^{7, 8}

Although it is well-documented that liver cancer is associated with constitutive inflammation and loss of HNF4 α , the relationship between these two events has only been recently investigated by Hatziaepostolou et al.⁹ Their findings suggest that inflammatory mediators such as IL-6 inhibit HNF4 α , while HNF4 α suppresses IL-6-STAT3 activation and liver inflammation by attenuating IL-6 receptor expression, thereby acting as a feedback

circuit to regulate hepatocarcinogenesis.⁹ This is the first study reporting that the HNF4 α -STAT3 axis governs hepatic transformation and oncogenesis, mechanistically linking inflammation and liver cancer. Moreover, this study further demonstrated that several miRNAs (such as miR-124, miR-24, and miR-629) tightly regulate this HNF4 α -STAT3 feedback circuit. Particularly, loss of HNF4 α results in IL-6 receptor-STAT3 activation via the inhibition of miR-124, while activation of STAT3 upregulates the hepatic expression of miR-629 and miR-24, which subsequently maintain the suppression of HNF4 α and sustain oncogenesis.⁹

In this issue of *Hepatology*, Ning et al. identified another important feedback loop in which the loss of HNF4 α leads to NF- κ B activation; this activation then further inhibits HNF4 α .¹⁰ This feedback circuit maintains the sustained suppression of HNF4 α and induces activation of NF- κ B, thereby promoting oncogenesis. Interestingly, the authors also demonstrated that several miRNAs play important roles in maintaining this feedback loop. Specifically, HNF4 α upregulates the expression of miR-124 and miR-7, which then attenuate NF- κ B activation. Conversely, NF- κ B activation induces the expression of miR-21, which subsequently downregulates HNF4 α expression.

MiRNAs, a group of small non-coding RNA molecules, play a role in the transcriptional and post-transcriptional regulation of a variety of genes that regulate cell differentiation, proliferation, survival, and transformation. In the liver, various miRNAs have been implicated in the pathogenesis of alcoholic liver injury, nonalcoholic fatty liver disease, viral hepatitis, fibrosis, and liver cancer.¹¹ The role of miRNAs in hepatocarcinogenesis has been actively investigated over the last decade. Emerging evidence suggests that the function of miRNAs in hepatic carcinogenesis is very complex. Different miRNA profiles in HCC have been reported from various studies, which is in part due to the different types of experimental approaches used to investigate miRNA expression in biological samples^{11, 12}. A large number of miRNAs have been shown to regulate hepatocarcinogenesis by affecting the expression of a wide variety of target genes that contribute to liver cell differentiation, growth, survival and apoptosis.¹³ Hatziapostolou et al. and Ning et al. identified a novel mechanism by which miRNAs regulate liver oncogenesis, which is mediated by maintaining the HNF4 α -inflammatory signal feedback loops (Fig. 1). This mechanism likely plays an important role in sustaining chronic liver inflammation during hepatocarcinogenesis, thereby promoting liver cancer progression.

Although the studies by Hatziapostolou et al. and Ning et al. nicely confirmed the regulation of the HNF4 α -STAT3 and HNF4 α -NF- κ B feedback loops by various miRNAs in cultured liver cancer cells *in vitro*, the interactions among miRNAs, HNF4 α , and inflammatory signals in the control of hepatocarcinogenesis *in vivo* are likely much more complex. For example, Hatziapostolou et al. demonstrated that HNF4 α inhibits STAT3 via the induction of miR-124 and, subsequently, by the inhibition of the IL-6 receptor. However, in addition to IL-6, many other cytokines can also activate STAT3 in hepatocytes and liver cancer cells,¹⁴ including various IL-6 family cytokines (such as leukemia inhibitory factor, ciliary neurotrophic factor, oncostatin M, cardiotrophin-1, and IL-11) and IL-22.¹⁴ Additionally, hepatocyte growth factor, epidermal growth factor, and hepatitis viral proteins can also activate STAT3 in hepatocytes, but to a lesser extent.¹⁴ It is not clear whether inactivation of

HNF4 α also enhances the STAT3 activation induced by these cytokines and mediators. In the HNF4 α -NF- κ B feedback loop, HNF4 α indirectly inhibits NF- κ B activity via the upregulation of miR-7 and miR-124 and subsequent suppression of RelA (a subunit of NF- κ B) expression.¹⁰ It is known that hepatic NF- κ B can be activated by many inflammatory cytokines (e.g., IL-1 and TNF- α), TLR ligands (e.g., LPS), and hepatitis B X protein.¹⁵ Therefore, it is likely that these mediators and the loss of HNF-4 α additively or synergistically promote NF- κ B activation in hepatocytes and liver cancer cells. Although Ning et al. concluded that miRNAs regulate the HNF4 α -NF- κ B feedback loop in hepatocytes and that NF- κ B promotes HCC metastasis, the actual role of NF- κ B in hepatocarcinogenesis is much more complex.¹⁵ First, NF- κ B may exert different effects in different cellular compartments, such as hepatocytes, nonparenchymal cells, and immune cells. Therefore, NF- κ B likely affects liver cancer development and progression via cell-specific mechanisms.¹⁵ It is not clear whether the miRNAs that regulate NF- κ B in hepatocytes also affect NF- κ B expression in nonparenchymal cells and immune cells or whether they subsequently modulate liver inflammation and carcinogenesis. Second, in addition to the upregulation of miR-124 and miR-7 by HNF4 α , many other miRNAs were also upregulated after overexpression of HNF4 α in HCC cells, of which several suppress HCC cell proliferation and contribute to the anti-tumor effect of HNF4 α .¹⁶ Finally, NF- κ B plays a wide variety of roles in regulating liver injury, inflammation, fibrosis, and regeneration, thereby indirectly affecting hepatocarcinogenesis.¹⁵

In summary, the study by Ning et al. described a novel mechanism by which miRNAs affect liver carcinogenesis via the regulation of the HNF4 α -NF- κ B feedback loop and is of clinical relevance for the prognosis of HCC patients. The authors showed that HCC patients with HNF4 α ^{high}RelA^{low} levels exhibit better overall survival and disease-free survival rates than those with HNF4 α ^{low} or HNF4 α ^{high}RelA^{high} levels, thereby suggesting that assessment of both HNF4 α and RelA levels yields better prognostic accuracy for HCC patients than HNF4 α alone. Additionally, several miRNA signatures have been identified that are useful for the diagnosis and prognosis of HCC patients.¹³ It would be interesting to determine whether the combined analysis of HNF4 α , RelA, and miRNAs exhibits greater prognostic accuracy than assessment of only HNF4 α and RelA. Finally, modulation of the HNF4 α -inflammatory signal feedback loop by targeting miRNAs may represent a novel strategy to treat and prevent HCC.

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Figure 1. A model depicting the regulation of HNF4α-inflammatory signal (STAT3 and NF-κB) feedback loops and hepatocarcinogenesis by miRNAs

HNF4α upregulates expression of miR-124 and miR-7, which then downregulates expression of RelA and subsequently inhibits NF-κB activation. Conversely, NF-κB activation induces the expression of miR-21, which consequently downregulates HNF4α expression. Moreover, HNF4α-associated upregulation of miR-124 also inhibits IL-6 receptor expression and subsequently attenuates STAT3 activation. On the other hand, STAT3 activation upregulates miR-24 and miR-629, which then attenuates HNF4α expression. Factors written in red mean those promote hepatocarcinogenesis while factors written in blue mean those inhibit hepatocarcinogenesis.