MicroRNA-feedback loop as a key modulator of liver tumorigenesis and inflammation

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Abstract

A recent work of Iliopoulos et al. published in Cell highlighted a circuit orchestrated by microRNAs (miRNAs) that results in liver tumorigenesis and inflammation. This feedback loop, governed by miR-24 and miR-629, promotes a hepatocyte nuclear factor-4α transient inhibition resulting in miR-124 induction and signal transducer and activator of transcription 3 activation. These promising data support the use of miRNA mimics or inhibitors as potent therapeutic approaches in liver cancer.

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Key words: Hepatocyte nuclear factor-4α; MicroRNA; Interleukin-6; Signal transducer and activator of transcription 3; Hepatocellular oncogenesis

COMMENTARY ON HOT TOPICS

Hepatocyte nuclear factor-4α (HNF-4α) belongs to the nuclear receptor superfamily of ligand dependent transcription factors NR2A1[1]. It is expressed in the kidney, intestine and pancreas. HNF-4α is most highly expressed in the liver[2-8], where it binds to the promoter of 12% of genes expressed in the adult liver[9]. It plays a key role in liver development during gastrulation[7] and orchestrates the parenchyma formation in the adult liver[8]. It also guides hepatoblasts to hepatocyte differentiation[9].

The conditional disruption of HNF-4α in mouse showed that HNF-4α regulates a number of genes involved in lipid, glucose, amino acid and xenobiotic metabolisms[4]. Related to its central role in liver physiology, HNF-4α has been reported to be involved in liver oncogenesis; however, its role is still controversial. A study by Xu et al[10] suggested that HNF-4α expression was increased in human hepatocarcinoma (HCC), which contrasted with other studies that reported that the HNF-4α level was decreased in HCC[11-13]. HNF-4α has also been associated with cancer and inflammation in the colon: its intestinal disruption in mouse revealed that HNF-4α protects against colitis and inflammatory disease[14,15] and promotes tumorigenesis[15]. Liver cancer is a clear example of an inflammation-related cancer[16-19]; therefore, it does not seem surprising that HNF-4α could also orchestrate an inflammatory program in the liver, as was shown last year by Wang et al[20] in experiments performed on hepatocarcinoma cells exposed to interleukin (IL)-6.

In a recent paper published in Cell, D. Iliopoulos’ Laboratory aimed to decipher the epigenetic circuit centered on HNF-4α, which promotes liver oncogenesis[21]. They particularly focused on microRNAs (miRNAs), which are small RNAs produced from non-coding DNA regions that control gene expression by binding to the 3’ untranslated region (3’ UTR) of target messenger RNAs (mRNAs). miRNA binding results in the degradation of the mRNA and/or the repression of its translation[22].
Since their discovery in 1994 in *Caenorhabditis elegans*, miRNAs have become an expanding area of research, and evidence supports the view that miRNAs are key regulators of many physiological processes, e.g., growth, proliferation and differentiation. They are also implicated in the initiation and progression of various cancers. Concerning HCC, microtranscriptomic analyses revealed a miRNA signature of liver cancer, i.e., miR-122 loss or miR-221 upregulation, and a number of studies support the view that miRNAs direct HCC progression through the induction of cell proliferation or metastasis and the suppression of apoptosis. Taken together, these data highlight miRNAs as promising diagnostic and therapeutic targets for HCC.

The work of Maria Hatziapostolou is a robust description of the molecular factors underlying HNF-4α loss-mediated hepatocarcinoma based on *in vitro* and *in vivo* experiments and confirmed on HCC human samples. Prior to the precise deciphering of hepatocarcinoma molecular origins, the authors confirmed that the silencing of HNF-4α was sufficient to increase cell colony formation and invasion of nontransformed immortalized human hepatocytes (IMH) and human liver cancer cell lines. On their own, the injection of IMH cells with a preliminary transient inhibition of HNF-4α in immunosuppressed mice resulted in tumor appearance. Interestingly, HNF-4α was maintained at a low level 55 d after cell xenografts, suggesting that transient silencing of HNF-4α initiated a feedback loop that stably maintained cell transformation and suppressed its own expression.

What are the factors involved in HNF-4α silencing? An miRNA screening was performed on a luciferase reporter gene containing the 3’ UTR region of HNF-4α, which revealed that miR-24 and, to a lesser extent, miR-629, were HNF-4α repressors. The transient expression of both miRNAs, the equivalent of HNF-4α silencing, transformed IMH cells, increased their invasiveness *in vitro* and promoted the appearance of tumors in mice. Strikingly, both miRNAs were induced following HNF-4α silencing, supporting the involvement of these miRNAs in the HNF-4α-dependent feedback loop.

Interestingly, miR-24 and miR-629 possess a binding motif for signal transducer and activator of transcription 3 (STAT3) in their promoters, a well-known effector of IL-6-dependent cancer inflammation (for a review), particularly in liver cancer (for a review). In brief, the binding of IL-6 to its receptor, IL-6R, activates the Janus kinase family, which phosphorylates STAT3 on tyrosine residues. STAT3 could then form homodimers before translocation into the nucleus to promote the expression of an inflammatory program (for a review). Here, the authors showed that in response to IL-6, STAT3 binding to miR-24 and miR-629 promoters increased, resulting in miRNA expression. In turn, miR-24 expression and HNF-4α silencing enhanced the phosphorylation status of STAT3, and thus its activation. STAT3 and IL-6 are two other members of the HNF-4α feedback circuit promoting liver oncogenesis; an antibody against IL-6 blocked all the HNF-4α silencing effects that they observed.

The next step consisted in screening of miRNAs possessing an HNF-4α binding site, which identified miR-124 as a preferential HNF-4α target. miR-124 was, in fact, an integral part of the feedback loop, because its inhibition by an antisense as-miR-124, or following HNF-4α silencing, enhanced STAT3 phosphorylation coupled to an induction of IL-6 secretion and expression of IL-6R, a direct target of miR-124.

The relevance of the loop miR-24/miR-629/HNF-4α/miR-124/STAT3 was confirmed *in vivo* with a mouse model of HCC induced by diethylnitrosamine. During tumor progression, the HNF-4α level was initially dropped off after four weeks, followed by miR-124 repression and inversely by miR-24 and IL-6R induction, which supports the essential role of the HNF-4α loop in tumor progression. One of the important finding of this study was that an injection of miR-124-mimic encapsulated into liposomes in this model was sufficient to limit tumor growth, even if this mimic was administered four weeks before analysis. miR-124 mimics were also able to prevent tumor development. Taken together, these results argue in favor of miR-124 delivery as a potent strategy for treating or preventing HCC. Although, to date, the efficacy of miRNA mimics have not been demonstrated *in vivo*, this approach could be attractive.

The prerequisite for this approach is that the complementary passenger RNA in the double stranded miRNA mimic does not create a new miRNA that could induce off-target effects.

The second exciting idea from this study relies upon the need for an inflammatory context to optimally disrupt the HNF-4α epigenetic circuit and promote tumorigenesis. If the tumors developed in a mouse model lacked STAT-3 in the liver, the tumors were smaller when the HNF-4α loop was less inhibited. This supports the hypothesis that HNF-4α loss engages an anti-tumorigenic program and a STAT3-dependent anti-inflammatory program, both orchestrated by the trio miR-24/miR-629/miR-124, to induce hepatocyte transformation. Such an epigenetic loop has already been described by the same authors for breast cancers, in which cell transformation was dictated by an epigenetic switch achieved by IL-6, nuclear factor-kB and the mRNA let-7.

Finally, the authors demonstrated the relevance of their model for human liver oncogenesis: more than 50% of HCC samples from patients showed a loss of HNF-4α and of miR-124, accompanied by an increase in miR-24 and IL-6R expression; all these parameters were associated with a higher level of phospho-STAT3 status in relation with IL-6 and IL-6R expression. As a confirmation of the HNF-4α circuit’s key role in HCC progression, the authors showed that the activity of the HNF-4α circuit becomes more deregulated as the HCC grade increased.

In summary, this work strongly argues for a tumor suppressor role of HNF-4α in liver, as was already sh-
of therapeutic agents currently used for HBV patients. HCC could also be performed using antioxidants, a class and, thus, reactivation of the HNF-4 

stress in the liver. In consequence, miR-24 inhibition associated with HCC development, contribute to oxidative damage. Recent studies have demonstrated that some miRNAs, particularly those deregulated in cancer, are encapsulated into exosomes and play a role as cell-cell mediators (41). In particular, miR-24 has been detected in exosomal structures, and a similar mechanism might exist in liver, permitting miR-24 to be delivered to hepatocytes by neighboring cells.

The most promising result of this study was that intravenous delivery of miR-124 mimic encapsulated into liposomes was able to prevent and reduce the growth of liver cancer. To date, few works have demonstrated the potency of an miRNA-based anticancer approach. However, the great advantage of HCC is that mimics or inhibitors against miRNAs are preferentially distributed to the liver (42). For that reason, the first miRNA-based therapy authorized in clinical trials is the anti-miR-122 compound Miravirsen, indicated for the treatment of hepatitis C virus (HCV), which uses miR-122 in hepatocytes for its replication. This clinical trial followed promising results obtained in primates infected with HCV and treated with an anti-miR-122 (43), which was well-tolerated (44). Concerning the HNF-4α circuit, the authors showed the efficiency of a mimic of miR-124 to perturb HCC development, but we could also conceive the injection of inhibitors against miR-24 and miR-629 to avoid HNF-4α loss. Even if miRNA inhibitor delivery appeared as a promising therapeutic approach, the use of these modulators poses a number of challenges. The question of treatment specificity could be raised. In fact, an miRNA could target multiple mRNAs (45,46), and, thus, many cellular pathways, pro- or anti-tumorigenic. It could be difficult to restrict the effect of one miRNA to only one target of interest. However, this notion could be generalized to many other treatments currently used, such as proteasome inhibitors or histone deacetylase inhibitors, which have demonstrated efficacy. Another major disadvantage could be the inhibition of a non-identified target. Their delivery could also be an important challenge, although many improvements have appeared recently, with chemical modifications limiting their degradation by nucleases (47). The other problem is that these inhibitors preferentially target the liver and other tissues are more difficult to enter (48). Finally, these inhibitors could also

Figure 1  From transient hepatocyte nuclear factor-4α inhibition to stable hepatocyte transformation. The transient inhibition of hepatocyte nuclear factor-4α (HNF-4α) following miR-24 and miR-629 induction promotes stable repression of the pro-tumorigenic HNF-4α feedback circuit. This results in miR-124 inhibition, followed by an increase in interleukin (IL)-6 secretion and IL-6R expression. These two events lead to hyperphosphorylation of signal transducer and activator of transcription 3 (STAT3), which auto-amplifies the process through the re-induction of miR-24 and miR-629.
be toxic. As suggested by the data of Hatziapostolou, mimics or inhibitors could be delivered in a free formulation, or protected into liposomes or even into exosomes, as was shown for siRNA. In conclusion, miR-124-based treatment could be a therapeutic strategy of choice for HCC, as an alternative to the anti-STAT3 approach. This therapeutic option has the great advantage of targeting the inflammatory, as well as the metabolic initiators, of HCC, and its efficiency demonstrated in this work argue for an epigenetic switch as the major event underlying cancer initiation.

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