MicroRNAs take on new roles in the diagnosis and treatment of hepatocellular carcinoma

MicroRNA Offers New Strategies in the Diagnosis and Treatment of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) has traditionally been an aggressive malignancy, with late diagnosis and a poor prognosis in most patients. However, recent advances in molecular diagnostics and treatment show promise in changing the HCC clinical landscape.

Researchers have made particularly promising discoveries in the area of miRNAs, the small, non-coding RNAs associated with regulatory functions in gene expression. MiRNAs associated with HCC can be used as biomarkers for the purposes of diagnosis and prognosis, and certain miRNAs have also been used as therapeutic targets.

As part of the molecular pathology of cancer, many genes are dysregulated, including genes that express miRNAs. Chromosomal rearrangements, methylation of promoters, and transcriptional induction are all capable of dysregulating miRNA expression[1].

In many cases, the miRNAs that are dysregulated are associated with oncogenes or tumor-suppressor genes, thereby contributing to a pro-oncogenic signaling milieu within the cell.

In the specific case of HCC, a number of miRNAs have been shown to be differentially regulated in the serum, plasma, and cancer cells of an affected individual. As such, many of these miRNAs show promise as biomarkers for the purpose of molecular diagnostics in the context of both HCC diagnosis and prognosis.

As described by He et al., early detection of HCC at a stage when the cancer is still surgically resectable, i.e. in a single nodule smaller than 2 cm, provides the best odds of patient survival[2].

Detecting HCC at such an early clinical stage is difficult using traditional diagnostic methods; thus, molecular diagnostics that make use of biomarkers represent a large unmet need in HCC care.

Although some molecular markers of HCC have already been described, such as serum α-fetoprotein (AFP), des-gamma carboxyprothrombin (DCP), and Dickkopf-1 (DKK1), these markers are neither specific for HCC nor present in all early-stage hepatocellular cancers[2].

As such, researchers have sought to identify novel biomarkers with enhanced specificity for HCC, whose presence can be detected reliably at the extreme early stages of HCC tumor progression. In addition, researchers have particularly sought circulating biomarkers for HCC, as these markers can be detected noninvasively.

Whereas differentially regulated miRNAs have been detected noninvasively in serum, plasma, and urine derived from HCC patients, the specificity and sensitivity of detecting these miRNAs has varied depending on the type of sample.

Samples derived from patient serum have thus far demonstrated the best sensitivity and selectivity profile, suggesting that serum-derived miRNAs may be the most useful in HCC early diagnosis[2].

Fortunately, two miRNAs — miRNA-21 and miRNA-199-a — have shown particular promise in early diagnosis of HCC. The expression of miRNA-21 is associated with reduced expression of the tumor suppressor phosphate and tensin homolog (PTEN) in HCC cells, whereas the expression of miRNA-199-a is associated with suppression of pro-oncogenic MAPK signaling.

Amr et al. found that miR-21 was significantly unregulated in the serum of HCC patients, while miR199-a was significantly downregulated in patient serum[3].

These investigators found that expression of miRNA-21 and downregulation of miRNA-199-a discriminated HCC patients from patients with chronic hepatitis ($P < .0001$ for each).

Notably, in this study, miR-21 upregulation was able to predict HCC with a sensitivity of 100% and specificity of 81.2%, whereas miR-199-a downregulation predicted HCC with a sensitivity of 54.5% and specificity of 100%. Clinical use of miRNA-21 and miRNA-199 expression to diagnose HCC will require that standardized threshold expression values be set.

Although miRNA-21 and miRNA-199 are useful in the early detection of HCC, these miRNAs are potentially less useful in monitoring the progression of HCC. Instead, another miRNA, miRNA-335, has been shown to be associated with HCC prognosis and treatment response.

Cui et al. determined that serum levels of miR-335 are associated with response to trans-arterial chemoembolization (TACE) in HCC, and that miR-335 expression tracked overall HCC prognosis[4].

These investigators found that miR-355 levels were significantly lower in HCC patients than in hepatitis patients or in healthy controls.

Low miR-335 expression levels were associated with HCC clinical features associated with tumor progression, including vascular invasion, cirrhosis, and larger tumor size, and low miR-335 expression also correlated with poorer prognosis and shorter overall survival. Notably, TACE response rate was elevated in those patients expressing relatively higher levels of miR-335.

Taken together, these results suggest miR-335 can serve as a molecular marker for aggressive HCC disease with poor prognosis. However, standardized thresholds for miR-335 expression have yet to be determined.

Other miRNAs that may be useful in detecting HCC include miR-101, which is downregulated in HCC patient serum, and miR-375, which is upregulated in patient serum[2].

The combination of upregulated miR-23b, miR-423, miR-375, miR-23a and miR-342-3p in patient serum is also strongly associated with HCC, as is the combination of miR-10a and miR-125b[2]. These miRNAs have also been variously associated with tumor size, invasive potential, grade, migration, and recurrence[2].

Aside from their diagnostic and prognostic potential, miRNAs serve as valuable therapeutic targets. Indeed, many miRNAs are intimately involved in regulating the expression of genes that contribute to oncogenesis, tumor progression, and loss of tumor suppressor capabilities within the cell.

One of the more promising miRNA therapeutic targets is miR-221, an oncogenic miRNA that is involved in growth, cell-cycle progression, cell proliferation, tumor invasion, and cell survival.

Moshiri et al. designed a “microRNA sponge” that inhibits miR-221 activity[5]. Their “miR-221 sponge” consists of adenovirus and adenovirus-associated viral vectors that drive expression of miR-221 binding sites, thereby sequestering miR-221 via competitive inhibition.

In HCC cell culture, these miR-221 sponges were able to significantly reduce endogenous levels of miR-221, commensurate with a rise in expression of the cell cycle-regulatory CDKN1B gene, a known target of miR-221.

Moshiri et al. also noted that miR-221 sponge expression in HCC cells had a pro-apoptotic effect. Although preliminary, these results with miR-221 sponges are promising and warrant further testing of safety and efficacy in treating HCC.

Another promising miRNA target is miR-122. In the liver, miR-122 plays a role in lipid metabolism, and it is highly expressed. In some instances, miR-122 is capable of acting as a tumor suppressor; miR-122 levels are reduced in HCC cells, and expression of miR-122 is capable of reversing the malignant phenotype of these cells.

However, miR-122 also plays an important role in the development of HCC from hepatitis C virus (HCV) infection[6]. As an important host factor for hepatitis C virus replication, miR-122 strongly promotes the accumulation of HCV RNA. Thus, miR-122 is an attractive therapeutic target in HCC that is associated with HCV infection.

An anti-miR that targets miR-122, miravirsen, is capable of producing prolonged decreases in HCV RNA levels in chronic hepatitis C patients, in a dose-dependent manner. Miravirsen is a locked nucleic acid anti-miR, comprised of a DNA and phosphorothioate backbone that stably and specifically binds to its target miRNA, preventing it from exerting any further regulatory effects.

Van der Ree et al. evaluated the virological response rate to the more traditional anti-HCV therapeutics peginterferon and ribavirin in patients dosed with miravirsen, assessing the long-term safety of miravirsen treatment in these patients[6].

In their retrospective follow-up study, van der Ree et al. observed sustained virological response in 7/12 patients with HCV genotype 1 who received miravirsen followed by peginterferon and ribavirin.

Among 27 patients who received miravirsen, there were no long-term safety issues or adverse effects over 35 months of follow up. Thus, anti-miR-122 therapy for HCV infection achieved via administration of miravirsen appears to be safe and effective; however, larger-scale clinical trials are necessary before any definitive conclusions are drawn.

Currently, the FDA requests a 5-year follow-up period for studies of anti-miR-122 therapies in humans because of the theoretical risk that HCC might be induced by lowered miR-122 expression.

Moving beyond simple targeting of miRNA, some novel therapeutics seek to directly modulate miRNA expression. Xiao et al. have described a small-molecule modulator of miR-34a that is capable of inhibiting HCC growth[7].

In a variety of cancer types, miR-34a serves as a tumor suppressor, inducing apoptosis and senescence and inhibiting cell proliferation. In HCC cells, miR-34a is typically severely downregulated or silenced completely.

Xiao et al. performed a screen of small molecules and identified a compound named Rubone (2’-hydroxy-2,4,4’,5,6’-pentamethoxychalcone) that rescued expression of miR-34a in HCC cell culture.

These researchers determined that Rubone did not inhibit growth in non-transformed human hepatocytes, suggesting that Rubone can restore the tumor suppressor function of miR-34a in cancerous cells without damaging healthy tissue.

The prospects of Rubone are particularly exciting given that Rubone showed similar anti-tumor activity to sorafenib, the frontline anti-HCC therapeutic, with seemingly less potential for systemic toxicity.

Notably, Rubone was effective in cells that expressed mutant or wild-type p53, but failed to restore miR-34a expression in cells that were p53 deficient. Xiao et al. therefore hypothesized that the pro-miR-34a activity demonstrated by Rubone is p53-dependent, and that Rubone achieves its effects on miR-34a expression by enhancing the DNA-binding or cofactor-recruiting function of p53 protein as it binds to the miR-34a promoter.

Taken together, the results described by Xiao et al. demonstrate that small molecules capable of modulating miRNA expression can serve as HCC therapeutic agents; however, these researchers performed their studies using only HCC cell culture and mouse xenograft models. These pre-clinical results warrant further investigation of the safety and efficacy of Rubone in human subjects.

Although many applications for miRNA in HCC have been described in the scientific literature, much of the existing work has been conducted at level of cell culture or xenograft models. The true clinical implications of miRNA are not yet fully understood.

In a recent review, Michele Ghidini and Chiara Branconi described many of the potential applications of miRNA in clinical practice, along with the inevitable limitations of miRNA technology[1].

For example, one major limitation of using serum miRNA levels to diagnose HCC is that standardization of serum miRNA assays is currently lacking. The interpretability of serum miRNA assays is hampered by spurious signal from the miRNAs of contaminating blood cells and difficulties in normalizing expression data between studies due to differing sample collection methodologies.

Thus, more, better standardized studies are warranted before many serum miRNAs can be taken into clinical practice as truly useful biomarkers.

Other limitations of miRNA technology that concern treatment of HCC have to do with the somewhat uncertain safety profile of modulating miRNA expression.

Although pre-clinical trials have demonstrated that modulating miRNA expression does not have deleterious effects on non-transformed cells in culture, the effects of modulating miRNA expression in a whole person remain somewhat unclear, and safety studies looking at systemic toxicity have, in large part, yet to be conducted.

Because miRNA are usually pleiotropic, influencing many, diverse phenotypes at once, modulating their expression can have unexpected effects on cellular physiology in systems entirely unrelated to HCC, and such effects must be carefully monitored before miRNA therapies can be used in the clinic.

Along similar lines, the delivery mechanisms used to introduce miRNA and anti-miRNA into cells may have toxicity issues in a whole organism that are unapparent in cell culture and xenograft-based studies.

For example, many miRNA targeting strategies make use of viral vectors, which may have immunostimulatory and pro-inflammatory effects when used in a whole person, thereby inducing serious adverse events.

Nonetheless, whereas the full implications of miRNA technology for clinical practice have yet to be worked out, studies and clinical trials in human patients are currently ongoing.

One such study is a phase I trial sponsored by Mirna Therapeutics to evaluate the safety of a liposomal formulation of miR-34, known as MRX34[8]. The study investigators are evaluating MRX34 in patients with unresectable primary HCC, as well as in patients with other advanced metastatic cancers. Based on the

hopeful results obtained with the miR-34a–enhancing agent Rubone, results from this human trial are highly anticipated by the scientific community[1].

Preliminary findings of the MRX34 trial, reported in April 2015 at the Annual Meeting of the American Association for Cancer Research, demonstrated that MRX34 had a reasonable safety profile in 23 HCC patients. Adverse events associated with MRX34 treatment were chiefly attributable to infusion reactions, and included fever, chills, and back pain, as well as gastrointestinal symptoms.

The HCC patients treated with MRX34 experienced a dose-dependent repression of oncogenes known to be miR-34 targets in circulating blood cells, which suggests that MRX34 may be efficacious in the treatment of various cancer types.

“We are very excited about the continued progress of our MRX34 program,” said Paul Lammers, M.D., President and Chief Executive Officer of study sponsor Mirna Therapeutics, in a press release[9]. “As the first microRNA mimic in clinical development, the data reported … represent substantial progress in demonstrating our ability to deliver miR-34 in a dose-dependent fashion into cells in patients and engagement of its biological targets.”

Although fraught with challenges, miRNA technology offers many opportunities in HCC diagnosis, prognosis, and treatment. For a condition that is frequently diagnosed very late and that offers few treatment options, miRNA provides a promising avenue of study.

Circulating miRNA biomarkers can allow for noninvasive diagnosis much earlier than other methods can offer, and therapeutics that modulate expression of key oncogenic and tumor suppressive miRNAs can complement the current, unsatisfactory standard of care, comprised of resection and sorafenib treatment.

Although few in number presently, future clinical trials studying safety and efficacy of miRNA technologies in the management of HCC are surely forthcoming.

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