REVIEW

Role of microRNAs in hepatocellular carcinoma: a clinical perspective

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Abstract: Hepatocellular carcinoma (HCC) is one of the most deadly tumors, and current treatments for the disease are often ineffective. The discovery of the involvement of microRNAs (miRNAs) in hepatocarcinogenesis represents an important area of investigation for the development of their clinical applications. These molecules may act as oncogenes or tumor suppressors by directly or indirectly controlling the expression of key proteins involved in cancer-associated pathways. On the clinical side, because of their tumor-specific expression and stability in tissues and in the circulation, miRNAs have been proposed as novel diagnostic tools for classification and prognostic stratification of HCC. In recent years, the therapeutic potential of miRNAs has been demonstrated in various preclinical studies. Anti-miRNA oligonucleotides and miRNA mimics have been found to have antitumor activity. Moreover, by exploiting tumor-specific expression of miRNA, efforts have been aimed at improving targeting of tumor cells by replicative oncolytic viruses while sparing normal cells. These areas are expected to be explored further in the upcoming years to assess the clinical value of miRNA-based approaches in HCC and cancer in general.

Keywords: hepatocellular carcinoma, microRNA, micromarkers, oncolytic viruses

Introduction

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is one of the most prevalent malignant diseases worldwide, and the third most common cause of cancer-related deaths.[1] In spite of the development of novel therapeutic strategies, the prognosis of advanced HCC remains poor, with a life expectancy of about six months from the time of diagnosis.[2] In most cases, HCC originates on a background of cirrhosis,[3] a chronic and diffuse hepatic disease that results from continuous liver injury and regeneration. Increased hepatocyte turnover, inflammation, and oxidative DNA damage are implicated in the pathogenesis of the disease. The prevalent risk factors for HCC are also the cause of liver cirrhosis, and include viral infections (eg, hepatitis B and C) and alcohol consumption; further risk factors include tobacco smoking, exposure to aflatoxin B1 and vinyl chloride, diabetes, and genetic disorders, such as hemochromatosis and alpha-1 antitrypsin deficiency.[4–9]

HCC is a cancer with a poor prognosis because of the low proportion of cases amenable to curative treatment at diagnosis and the high rate of recurrence following therapeutic intervention. The estimated recurrence rate can be as high as 70%–80% at five years, considering both true recurrences and HCC de novo, and this contributes significantly to the dismal prognosis of HCC. In addition, traditional therapies are not effective for HCC and are too toxic for patients with cirrhosis. Transarterial chemoembolization and

radioembolization are the main treatments for intermediate-stage HCC at the present time.[10] The only systemic therapy available for advanced HCC is based on the multikinase inhibitor sorafenib,[11] which is the most effective therapeutic tool for advanced nonresectable HCC, in which it can slightly improve patient survival.[12,13] The survival of patients with advanced HCC treated with sorafenib depends on the absence of liver dysfunction and on the status of the patient.[14,15] In the past few years, use of sorafenib in combination with transarterial chemoembolization has significantly improved survival rates in patients with advanced HCC.[16,17] New perspectives in cancer treatment have appeared recently with the advent of the microRNAs (miRNAs), a novel class of noncoding small RNAs.

microRNAs

microRNAs are short RNAs (containing 20–24 nucleotides) that play an important role in all biological processes by post-transcriptional regulation of protein-coding genes.[18] They constitute a large class of phylogenetically conserved genes, with more than 2000 miRNAs having been discovered in humans.[19] miRNAs are transcribed by RNA polymerase II to produce a primary pre-miRNA, cleaved by the Drosha-DGCR8 complex to a shorter pre-miRNA approximately 70 nucleotides long. The pre-miRNA is transported by Exportin-5/RanGTP from the nucleus to the cytoplasm, where it is processed to a short miRNA-miRNA*-duplex by the Dicer-TRPB complex. The *strand is usually degraded, and the other strand becomes the mature miRNA that is incorporated into the RNA-induced silencing complex.

This ribonucleoprotein complex eventually becomes bound to regions of homology present in messenger RNAs (mRNAs), usually within their 3′ untranslated regions. Recruitment of the RNA-induced silencing complex to the target mRNA can promote either degradation or repression of translation. Via these mechanisms, each miRNA can modulate the expression of target protein-coding genes (reviewed recently by Davis-Dusenbery and Hata[20] and Farazi et al[21]). Each miRNA can promote the targeting and modulation of expression of tens or even hundreds of mRNAs. On the other hand, each mRNA can be targeted and under the control of several miRNAs. It has also become evident that mRNAs or RNAs highly expressed by pseudogenes or long noncoding RNAs can act as a "sponge" to reduce interaction between miRNAs and other mRNA species. Therefore, the network of interactions between mRNA and miRNA is complex and largely still to be clarified in a comprehensive systemic fashion.

Role of miRNAs in cancer

In view of their role in regulating the expression of protein-coding genes, miRNAs now have a widely recognized role in human carcinogenesis. Initial evidence came from detection of their aberrant expression in all human cancers.[22] Some of them are frequently found to be upregulated or downregulated in cancer in comparison with normal tissue. There is now mounting experimental evidence indicating that they may act as oncogenes or tumor suppressors by disrupting regulation of genes encoding for oncoproteins and tumor suppressor proteins.[23]

In vivo models have provided conclusive proof that miRNAs play a key role in tumorigenesis. Proof of principle of the involvement of miRNA in the development of neoplastic disease was provided by generation of the Eμ-miR155 transgenic mouse that develops a lymphoproliferative B-cell malignancy.[24] Further animal models confirmed that deregulated miRNAs could be involved in tumorigenesis in vivo, mostly in hematologic malignancies.[25–28] Targeted deletions demonstrated this functional activity in putative tumor suppressor miRNAs; for example, a miR-15a/miR-16-1 knockout mouse model is predisposed to development of an indolent form of leukemia, resembling human chronic lymphocytic leukemia, where deletion of these miRNAs is found in over 60% of cases.[29]

Role of specific miRNAs in HCC

Involvement of miRNAs in HCC has been demonstrated, as in other cancers. HCC develops via deregulation of various molecular pathways, including p53, RAS/MAPK, PI3K/AKT/mTOR, WNT/β-catenin, MET, MYC, and transforming growth factor beta. Genetic and epigenetic alterations, as well as aberrant miRNA expression, can affect these crucial cancer-associated pathways (for a detailed review, see Negrini et al[30]). Several studies have shown that expression of miRNA is deregulated in HCC in comparison with normal liver tissue (again, see Negrini et al[30] for a comprehensive review). In light of reports from independent studies, consistent deregulation of miR-122, miR-199, miR-221, and miR-21 appears to be particularly important in HCC (Figure 1). Interestingly, both miR-122 and miR-199a are among the miRNAs most highly expressed in the normal liver.[31]

miR-122 is unique among the deregulated miRNAs, in that it is almost exclusively expressed physiologically in the adult liver,[32] where it appears to act as a key regulator of the differentiation of adult hepatocytes via repression of genes not specific to the liver.[33–35] At the molecular level, this effect is achieved by regulation of CUTL1, a transcriptional repressor
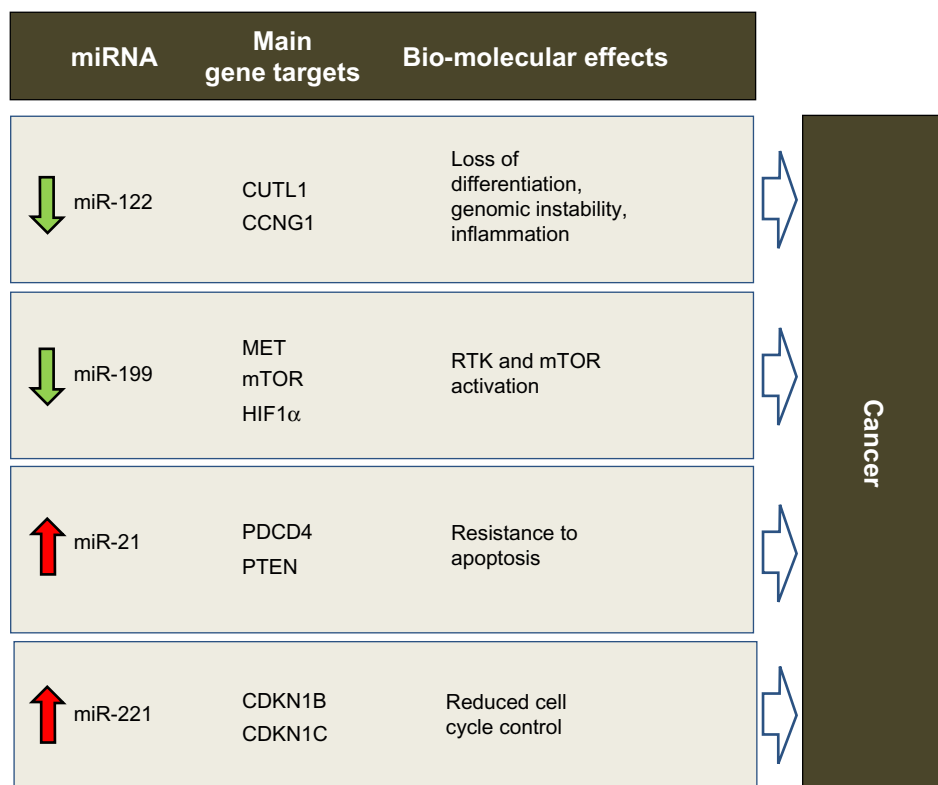
Figure 1 Predicted effects of deregulated miRNAs in hepatocarcinogenesis. miR-122, miR-199, miR-221 and miR-21 were shown by various studies to be consistently deregulated in HCC. Via aberrant control of protein-coding gene targets (only the major ones are shown), their deregulation is expected to induce biomolecular effects that promote tumorigenesis.
Abbreviations: miRNA, microRNA; HCC, hepatocellular carcinoma.

of genes specifying terminal differentiation in multiple cell lineages, including hepatocytes. CUTL1 was shown to be the most prominent repressed target of miR-122.[33] In HCC, miR-122 is downregulated in approximately 70% of cases, suggesting a tumor suppressor function for this miRNA. Various lines of evidence now support this hypothesis. Enforced expression of miR-122 can induce apoptosis and arrest of the cancer cell cycle,[36,37] inhibit tumorigenicity in liver cancer cell lines in vivo,[36] and sensitize cells to sorafenib or doxorubicin.[36,38] In addition, loss of miR-122 expression in patients with liver cancer is correlated with the presence of metastasis[39,40] and a shorter time to recurrence.[38] The role of miR-122 in liver cancer has been demonstrated directly by the generation of miR-122 knockout mice.[41,42] These mice were characterized by hepatic inflammation, fibrosis, and development of spontaneous tumors similar to HCC, demonstrating the tumor-suppressor function of this miRNA and its important role in liver metabolism and differentiation of hepatocytes. These phenotypic effects could at least in part be understood by identification of miR-122 gene targets. Reduced control of CUTL1, as previously mentioned, may be responsible for the lack of differentiation that characterizes HCC cells. Another known target of miR-122 is cyclin G1,[43]

which is a negative regulator of p53 and is frequently upregulated in HCC. In a mouse model, the absence of cyclin G1 was associated with less susceptibility to developing liver tumors.[44] Reduced levels of miR-122 could lead to inhibition of p53 activity by increasing cyclin G1 levels.[38] Loss of miR-122 could also directly affect the intrinsic apoptotic pathway by reduced regulation of the antiapoptotic protein, Bcl-w.[45] miR-122 invasive and metastatic properties were instead linked to loss of control on ADAM17 (a disintegrin and member of the metalloproteinase family).[39] By targeting ADAM17, miR-122 can reduce in vitro migration and invasion, in vivo tumorigenesis and angiogenesis, and local invasion in the livers of nude mice. A similar effect could also be expected when targeting ADAM10.[36]

miR-199 has been reported to be consistently downregulated in the majority of HCC,[43,46,47] suggesting a tumor suppressive function. All three members of the miR-199 family, ie, miR-199a-1, miR-199a-2, and miR-199b, have emerged as being frequently downregulated in HCC. Phenotypically, enforced expression of miR-199a in HCC cells leads to cell cycle arrest at G1 phase, reduced invasive capability, and enhanced susceptibility to hypoxia. In patients with HCC, downregulation of miR-199a was associated with a higher

recurrence rate and shorter time to recurrence after surgery. These effects could be explained by modulation of target genes, such as MET, mTOR, and HIF-1α.[48–51] Another important target of miR-199 in HCC is CD44, a transmembrane glycoprotein involved in cell-cell interaction, cell adhesion, and migration.[52] Further, absence of control over Discoidin domain receptor-1 tyrosine kinase may promote cell invasion processes in HCC.[53]

Among the miRNAs that are upregulated in HCC, there is evidence in support of the tumor-promoting activity of miR-221. It is upregulated in 70%–80% of HCC samples.[54] From a functional point of view, HCC cells overexpressing miR-221 show increased growth, proliferation, migration, and invasion capability.[54–56] miR-221 antagomirs inhibit growth of liver cancer cells, and enforced miR-221 expression was shown to enhance tumorigenesis of cells when implanted in mice. In this setting, overexpression of miR-221 promotes tumor progression and shortens the survival of the animal.[57] More recently, a transgenic mouse model characterized by overexpression of miR-221 in the liver was developed. This model demonstrates high susceptibility to HCC in male animals, which can be partly inhibited by challenge with anti-miR-221 oligonucleotides.[58] By modulating multiple gene targets, miR-221 has been shown to affect several cancer pathways. The cell cycle could be promoted by modulation of the cyclin-dependent kinase inhibitors, CDKN1B/p27 and CDKN1C/p57.[54] Other important targets include the BH3-only protein, Bcl2-modifying factor[59] (a proapoptotic protein) and PTEN (a negative regulator in the PI3K-AKT-mTOR pathway).[56] DNA damage-inducible transcript 4, another negative regulator of the mTOR pathway, was also identified as a target of miR-221.[57] miR-221 was also shown to affect invasion and metastasis by controlling TIMP3, a tissue inhibitor of metalloproteases.[56] These examples outline the importance of deregulation of even a single miRNA in cancer. miR-221 is a paradigmatic example of an miRNA regulating multiple pathways at one time.

miR-21 is a potent oncogene when upregulated. It is overexpressed in HCC as well as in several other human malignancies, including breast, colon, lung, pancreas, prostate, and stomach cancers.[60] Overexpression of miR-21 in cultured human cells can protect against apoptosis[61] and increase tumor cell proliferation and migration.[62] In vivo, miR-21 inhibition suppressed cell proliferation and increased apoptosis in a cancer xenograft model.[62] In a transgenic mouse model, overexpression of miR-21 led to a pre-B malignant lymphoma that regressed completely when miR-21 was inactivated, partly as a result of apoptosis.[28] Another

important activity of miR-21 is chemoresistance induced against a variety of anticancer compounds. These multiple effects are linked to several genes targeted by miR-21 (for a recent review describing miR-21 target genes, see Buscaglia and Li[63]). Among the most important of these is PTEN,[64] which promotes cell survival via activation of the PI3K-AKT pathway, and tumor suppressor programmed cell death 4,[65,66] a protein believed to have a role in apoptosis induced by transforming growth factor-beta.

Clinical implications
miRNA diagnostics

Profiling of miRNA expression could be a useful tool for classification purposes and for improving prognostic stratification. Previous reviews have summarized the potential applications of miRNAs as diagnostic and prognostic markers in human cancer and in particular liver cancer.[30,67,68]

Several miRNAs may have potential prognostic significance. Table 1 summarizes the presently available data. For classification purposes, it is shown that miR-200c, miR-141 and miR-126, alone or in combination, could be used to distinguish primary HCC versus other tumor metastases to the liver with very high accuracy; moreover, the ratio of miR-205 to miR-194 expression could be used to distinguish between gastrointestinal tumors and metastases outside the gastrointestinal system,[69] which is important considering that the liver is the main metastatic site for gastrointestinal tumors.[70]

An emerging area of investigation with regard to miRNAs is their potential use as circulating biomarkers. Because of their different levels in the serum or plasma of patients affected by a range of diseases in comparison with healthy subjects,[71] miRNAs could be useful biomarkers for patient follow-up.[72] Table 2 summarizes the studies of miRNAs in serum and plasma, confirming the potential use of miRNAs as sensitive markers for detection of an underlying HCC and for prognostic stratification of the disease. Among the miRNAs, miR-122 and miR-21 levels have been reported by more than one study to be significantly higher in patients with HCC. Because increased levels are also detected in chronic hepatitis, their usefulness as clinical tumor markers needs to be validated further.[73–77]

Overall, these results point to miRNAs being potential biomarkers that could improve our ability to stratify the prognosis and monitor follow-up in patients with HCC. Their stability in formalin-fixed and paraffin-embedded samples as well as in body fluids like serum or plasma is an important property for enabling their detection and quantification in biological samples, which are frequently used in clinical

Table 1 microRNAs with potential prognostic impact in patients with HCC

miRNAs	Molecular alteration	Clinical significance	Reference
20 miRNAs	Signature	Venous metastasis, overall survival	131
19 miRNAs	Signature	Poor survival	47
miR-19a, miR-886-5p, miR-126, miR-223, miR-24 and miR-147	Signature	Predictor of overall survival and recurrence-free survival after LT	132
miR-26a	Downregulation	Poor survival	133
miR-122	Downregulation	Gain of metastatic properties	39,40
miR-122	Downregulation	Early recurrence	38
Let-7 members	Downregulation	Early recurrence	134
miR-199a-3p	Downregulation	Reduced time to recurrence	49
miR-199b-5p	Downregulation	Poor overall survival and progression-free survival rates	51
miR-101	Downregulation	Advanced tumor progression, poor prognosis	135
miR-125a	Upregulation	Better survival	136
miR-92, miR-20, miR-18	Upregulation	Poor differentiation	46
miR-372	Upregulation	Advanced TNM stage	137
miR-221	Upregulation	Multinodularity, reduced time to recurrence	59
miR-221	Upregulation	Gain of metastatic properties	138
miR-221	Upregulation	High tumor capsular infiltration	139
miR-17-5p	Upregulation	Multiple tumor nodules, vein invasion, shortened overall survival	140
miR-155	Upregulation	High recurrence and poor prognosis following OLT	141
miR-203	Upregulation	Good prognosis	142
miR-18	Upregulation	Poor prognosis	143

Abbreviations: HCC, hepatocellular carcinoma; miRNAs, microRNAs; TNM, tumor-node-metastasis; LT, liver transplantation; OLT, orthotopic liver transplantation.
Note: Data adapted from Negrini et al.[30]

assays. However, at present, prospective studies are still required before miRNAs can be used in the clinic as biomarkers for cancer.

miRNA therapeutics

miRNA inhibition

In the past few years, several lines of evidence have indicated that strategies based on modulation of miRNA activity could be a novel approach to treating cancer (Figure 2). In 2005, Krutzfeldt et al showed that intravenous administration of specific antagomirs could silence miR-122 in the mouse liver.[78] A few years later, Elmen et al demonstrated that inhibition of miR-122 by administration of anti-miRNA oligonucleotides in nonhuman primates was a promising approach for reducing miRNA activity in the adult liver without any evidence of toxicity.[79] These proofs of principle established the basis for the various studies that have been performed in cancer models in vivo.

Anti-miR-221 was shown to have antitumor activity, which was demonstrated by intratumoral injections of anti-miRNA oligonucleotides into prostate carcinoma cell-derived tumors,[80] in melanoma cell xenotransplants,[81] and in multiple myeloma xenografts.[82] Park et al showed the ability of anti-miR-221 molecules to reduce proliferation of tumor cells and promote survival in an orthotopic mouse model of HCC.[83] Anti-miR-221 was also shown to downregulate miR-221

levels in the liver of the miR-221 transgenic mouse and to achieve a significant reduction in the number and size of tumors in comparison with untreated animals.[58]

The role of the miR-21 oncomir was investigated using anti-miR-21 molecules in vivo. Use of anti-miR-21 led to complete regression of pre-B lymphoid-like malignancies in mice overexpressing miR-21.[28] Anti-miR-21 was also reported to have significant antitumor activity in SCID mice bearing human multiple myeloma xenografts.[84] Thus, by confirming the feasibility as well as short-term safety and efficacy of these molecules in large-scale preclinical settings, these studies established the basis for the use of anti-miRNAs in clinical trials.

The first miRNA-targeted drug, a molecule known as miravirsen SPC3649, has been used in various Phase I investigations and is currently in a Phase II clinical trial for the treatment of hepatitis C virus (HCV) infection.[85] This trial stems from the discovery of involvement of miR-122 in HCV RNA accumulation, and demonstrated that treatment of chronically infected nonhuman primates with an LNA-modified anti-miR-122 oligonucleotide was well tolerated and led to long-lasting suppression of HCV viremia.[86,87]

miRNA replacement

In addition to inhibition of oncomirs, another approach to treating cancer is based on restoration of tumor suppressor miRNAs. Several examples of this approach

Table 2 Circulating microRNAs in liver disease

miRNAs	Sample	Clinical condition	Clinical relevance	Reference
miR-122	Serum	High levels in patients with HCC or chronic hepatitis; lower levels in severe stages of fibrosis	Biomarker for liver injury but not specific for HCC; indicator of fibrosis progression in CHC infection; marker to distinguish patients with CHC from healthy controls	74,76,77, 144,145
miR-21	Serum/ plasma	High levels in patients with HCC or chronic hepatitis; higher levels in patients with HCC than in those with chronic hepatitis or healthy volunteers	Biomarker for liver injury but not specific for HCC; biochemical marker for HCC	73–75,146
miR-223	Serum	High levels in patients with HCC or chronic hepatitis	Biomarkers for liver injury but not specifically for HCC	73
miR-885-5p	Serum	High levels in patients with HCC, LC, or CHB	Complementary biomarker for detection and assessment of liver pathologies	147
miR-16 miR-34a	Serum	Higher levels in patients with NAFLD than in controls	Correlation with liver enzymes levels, fibrosis stage, and inflammation activity; biomarkers of diagnosis and histological disease severity in patients with CHC or NAFLD	74
miR-221	Serum	High levels correlated with tumor size, cirrhosis, and tumor stage	Predictive significance for prognosis of patients with HCC	146
miR-15b miR-130b	Serum	Higher levels in tumors during the exploration phase on resected tumor/adjacent and nontumor tissues; lower levels after surgery	Biomarker with clinical value for HCC screening	148
miR-20a miR-92a	Plasma/ serum	High levels in HCV-infected patients with fibrosis compared with healthy volunteers or liver disease not associated with HCV; higher levels in acute and chronic HCV-infected patients as compared with healthy volunteers	Biomarkers for early detection of HCV infection; miR-20a is a predictive biomarker of HCV-mediated fibrosis	149

Abbreviations: HCC, hepatocellular carcinoma; CHC, chronic hepatitis C; CHB, chronic hepatitis B; LC, liver cirrhosis; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus; miRNAs, microRNAs.
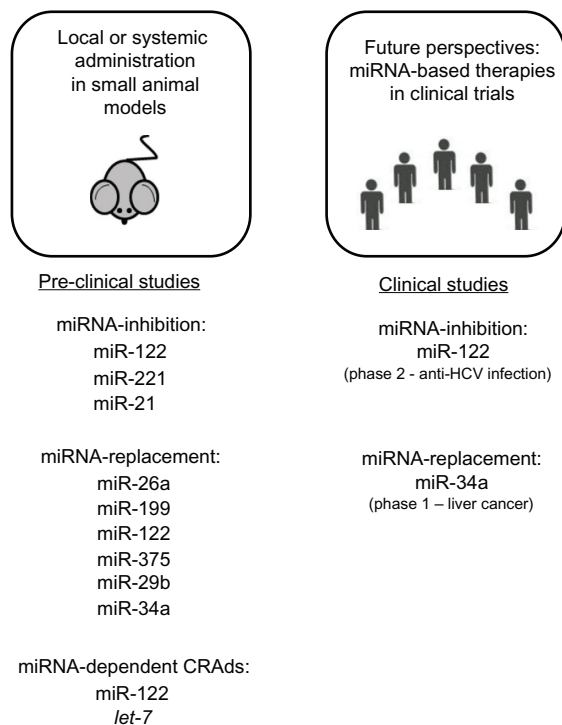

Figure 2 Therapeutic strategies based on modulation of miRNA activity. Summary of preclinical studies (on the left) based on miRNA inhibition, miRNA replacement, and conditionally replicating adenoviruses regulated by miRNA (microRNA) target elements. On the right are clinical trials ongoing using miRNA-based drugs.

already exist. Enforced expression of miR-26a using an adenoassociated (AAV8) delivery system inhibited tumorigenicity in a *myc* mouse HCC model.[88] Both AAV8 miR-199 and cholesterol conjugated small RNA delivery systems could effectively restore miR-199a/b-3p and reduce tumor size in HCC xenografts.[31] The tumor suppressor role of miR-122 in HCC was confirmed by strong inhibition of tumorigenesis using AAV-mediated delivery of miR-122 in a *myc* mouse HCC model.[41] Administration of cholesterol-conjugated 2′-O-methyl-modified miR-375 mimics significantly suppressed growth of hepatoma xenografts in nude mice.[89] miR-29b could sensitize HCC cells to various apoptotic signals and could suppress the ability of HCC cells to form tumors in nude mouse xenograft models.[90]

Further studies demonstrating the antitumor effectiveness of miRNAs have been reported in other types of tumors and experimental settings.[91–93] An important example is restoration of miR-31, the action of which could alter the invasive properties of disseminated tumor cells, raising the possibility of developing miRNA-based strategies for the treatment of metastatic disease.[94]

The above studies establish miRNAs as promising molecules in cancer therapy. In this context, miR-34a is the first miRNA mimic to reach the clinic (http://clinicaltrials.gov/

ct2/show/NCT01829971).[95] Earlier studies showed that lentivirus expressing miR-34a could prevent tumor formation and progression in mouse models of lung adenocarcinoma induced by K-ras and p53.[96] Inhibition of tumor growth and increased survival were also observed in mice bearing multiple myeloma xenografts treated with miR-34a mimics.[97] Very recently, expression of miR-34 combined with the cytokine interleukin-24 showed synergistic antitumor activity in a xenograft model of HCC, indicating the possibility of using a multiple-armed miRNA-based viral vector in cancer therapy.[98]

Oncolytic viruses

Oncolytic viruses are developed to replicate selectively in tumor cells. They are engineered to have cytotoxic effects in tumor cells with minimal toxicity in normal cells. For this reason, they hold promise for the treatment of cancer.[99–101] The first conditionally replicating adenovirus (CRAd), known as ONYX-015, carried a deletion in the E1B-55 kDa coding region, which was designed to limit its replication with cells having dysfunctional p53.[99] It was used in clinical trials either alone or in combination with chemotherapeutic agents.[102–107] Since then, progress has been made in this field, with more selective and potent oncolytic viruses having been entered into clinical trials (see Patel and Kratzke[108] for a recent review).

Among the oncolytic viruses in use, the presence of the gene for the immune stimulator granulocyte-macrophage colony-stimulating factor (GM-CSF) is improving significantly antitumor activity. The safety and biological activity of an oncolytic viral vector based on herpes simplex virus type-1, known as OncoVEX[GM-CSF] (Amgen, Thousand Oaks, CA, USA), has been assessed in several clinical trials.[109,110] Oncolytic adenoviruses armed with GM-CSF have also been used successfully in the treatment of patients with advanced metastatic tumors refractory to conventional therapies. Their use resulted in antitumor immunity and increased median overall survival.[111–113] Oncolytic poxvirus carrying GM-CSF was also investigated in clinical trials, and demonstrated an oncolytic and immunotherapeutic mechanism of action, tumor responses, and dose-related survival in treated patients with HCC.[114–116]

To generate safer oncolytic viruses, miRNA-mediated suppression of virus replication has been used successfully to reduce pathogenic effects in normal tissue. The cellular tropism of a picornavirus was modulated by engineering target sequences for muscle-specific miRNAs into the viral genome, thereby avoiding development of lethal myositis in tumor-bearing mice.[117] Ylosmaki et al developed a new type of conditionally replicating CRAd regulated by miR-122 target elements within the 3′ untranslated regions of the E1A gene, achieving liver-specific suppression of viral replication and reducing hepatotoxicity.[118] At the same time, it was found that viral oncolytic activity was not damaged in targeted tumors in vivo.[119] A similar approach has been reported by Cawood et al, who showed that incorporation of miR-122-binding sites to control E1A mRNA significantly reduced adenoviral replication and liver toxicity in mice.[120,121] Another approach to E1A regulation combines an miR-122 control with chromogranin-A gene promoter-controlled virus replication, allowing use of high doses of adenovirus for more effective tumor treatment with limited liver toxicity.[122] Other miRNAs involved in the control of oncolytic virus replication include a let-7-dependent oncolytic adenovirus, which is able to replicate only in cells lacking miRNA expression, such as HCC cells, and not in normal liver cells.[123]

These reports have established the potential value of engineered oncolytic viruses in the treatment of human malignancy. In this context, the target sequences of miRNAs could ensure the detargeting from normal tissues of virus replication, which still remains active in tumor cells.

In human cancer, most of the malignant cells are unable to produce tumors when implanted into immunodeficient mice. Still, 1%–2% of these cells maintain cell renewal capabilities and may generate tumors. It has been suggested and demonstrated in several instances that these cells persist in tumors as distinct populations, which are designated as tumor-initiating cells or cancer stem cells.[124] Cancer stem cells are essential for the growth of solid tumors and hematologic malignancies, as well as for seeding of metastases. Because they consist largely of nonproliferating cells, they are also intrinsically resistant to traditional therapies, thereby being responsible for relapses following therapy. Hence, it has been suggested that effective therapies against cancer stem cells could potentially lead to complete eradication of tumors.

Several studies have demonstrated the importance of miRNAs in the control of stem cells[125,126] as well as the phenotype of the cancer stem cell.[124] In HCC, identification of a cell population called CD133+ which has the characteristics of cancer stem cells, has provided new perspectives for characterization of liver cancer.[127] Several lines of evidence indicate that aberrant expression of miRNAs may control and lead to maintenance of liver tumor-initiating cells through aberrant modulation of stem cell-associated genes.

Table 3 microRNAs and cancer stem cells in HCC

miRNAs	Regulatory role in liver cancer stem cells	Reference
miR-181	Maintenance of EpCAM + AFP + HCC cells by inhibiting hepatic cell differentiation and promoting HCC stemness through targeting the transcriptional regulators CDX2 and GATA6 as well as the Wnt signaling inhibitor, NLK	150,151
miR-193b	Regulation of proliferation, migration, and invasion potential of HCC cells by downregulation of ETS1	152
miR-130b	Promotion of tumor growth and self-renewal in CD133+ T-ICs by downregulation of TP53INP1	153
miR-122	Modulation of self-renewal of hESCs and HCC proliferation by suppressing translation of the metabolic protein PKM2	154
miR-145	Modulation of downstream stem cell-related gene target Oct4	155
miR-150	Self-renewal of CD133+ liver CSCs through direct negative regulation of the downstream target, c-Myb	156
miR-200c	The miR-200-controlled epithelial-mesenchymal transition is functionally important for the development of stem-like cells associated with poor prognosis	157
miR-216a; miR-216a/217	Overexpression of these miRNAs activates the PI3K/Akt and TGF-β pathways by targeting PTEN and SMAD7, contributing to neoplastic transformation of LPCs to hepatic T-ICs, facilitating hepatocarcinogenesis and tumor recurrence in HCC	158,159
miR-214	Silencing of miR-214 modulates expression of EZH2, CTNNB1, and CDH1, increasing EpCAM+ stem-like cells	160

Abbreviations: HCC, hepatocellular carcinoma; EpCAM, epithelial cell adhesion molecule; AFP, alpha-fetoprotein; CDX2, caudal type homeobox transcription factor; GATA6, GATA binding protein 6; NLK, nemo-like kinase; T-ICs, tumor-initiating cells; PKM2, pyruvate kinase isozymes M1/M2; CSCs, cancer stem cells; TGF-β, transforming growth factor-beta; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SMAD7, mothers against decapentaplegic homolog 7; LPCs, liver progenitor cells; EZH2, enhancer of zeste homolog 2; CTNNB1, β-catenin; CDH1, E-cadherin; miRNAs, microRNAs.

Table 3 summarizes the findings in this area. As indicated earlier, miRNA-based approaches are potentially feasible. Targeting of cancer stem cells using an miRNA-based approach represents a novel area of investigation aimed at eradicating liver tumor-initiating cells, thereby treating HCC.[128–130]

Conclusion

While miRNA-based approaches are not presently used in the clinic, their potential applications are expanding in various areas of interest. Prognostic stratification, follow-up monitoring, and innovative therapeutic approaches are areas that might benefit from use of miRNAs. It should be noted that it has only been during the last 10 years that investigation of miRNAs in cancer has been initiated, and many more studies are needed to move this field forward into the clinical setting. Validation based on prospective studies of the use of miRNAs as cancer biomarkers is needed. Application of miRNA replacement or inhibition approaches need larger preclinical studies to assess their potential efficacy in specific contexts. The present knowledge, as summarized here, should form the basis of studies aimed at development of miRNA-based clinical applications.

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Disclosure

The authors report no financial conflicts of interests in this work.

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