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MiR-22 as a metabolic silencer and liver tumor suppressor

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Abstract

With obesity rate consistently increasing, a strong relationship between obesity and fatty liver disease has been discovered. More than 90% of bariatric surgery patients also have non-alcoholic fatty liver diseases (NAFLDs). NAFLD and non-alcoholic steatohepatitis (NASH), which are the hepatic manifestations of metabolic syndrome, can lead to liver carcinogenesis. Unfortunately, there is no effective medicine that can be used to treat NASH or liver cancer. Thus, it is critically important to understand the mechanism underlying the development of these diseases. Extensive evidence suggests that microRNA 22 (*miR-22*) can be a diagnostic marker for liver diseases as well as a treatment target. This review paper focuses on the roles of *miR-22* in metabolism, steatosis, and liver carcinogenesis. Literature search is limited based on the publications included in the PubMed database in the recent 10 years.

Keywords

MicroRNA-22(miR-22); Cancer; Liver; Metabolism; Steatosis; Non-alcoholic steatohepatitis; Hepatitis

1. Introduction

MicroRNA 22 (*miR-22*) is highly conserved across vertebrate species and its expression is ubiquitously expressed in various organs.[1–5] The *miR-22* gene is located on chromosome 17p13, its cDNA catalyzed by RNA polymerase II is ~1.3 kb. In addition, its transcription

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Author's contributions

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Declaration of competing interest

The authors declare that there is no conflict of interest.

start site lacks TATA box.[6] Many studies have revealed that *miR-22* is implicated in the development of various types of cancer including liver, colon, prostatic, breast cancer, gastric cancers, and many others. In general, *miR-22* is considered as a metabolic silencer and a tumor-suppressor. However, its oncogenic effect has been documented as well. Furthermore, *miR*-22 has many biological functions including inflammatory and immune regulation; arterial smooth muscle cell proliferation and migration regulation; and cardiac and vascular remodeling.[7–9] In this review paper, we summarize the role of *miR-22* in liver disease development.

2. *MiR-22* as a metabolic silencer

MiR-22 is an important regulator of dyslipidemia. It has been shown that *miR-22* deficiency prevents high-fat diet (HFD)-induced dyslipidemia by inhibiting the expression of genes (sterol regulatory element binding protein-1 (*Srebp-1*), CC motif chemokine ligand 2 (*Ccl2*), interleukin 6 (*Il-6*), and interferon gamma (*Ifng*). Thus, *miR-22* promotes lipogenesis and inflammation.[10]

MiR-22 along with *miR-34a* are up-regulated in the liver of diabetic db/db mice. *miR-22* reduces the levels of E1A binding protein p300 (Ep300) as well as transcription factor 7 (Tcf7), and *miR-34a* decreases the protein level of its target gene Wnt Family Member 1 (*Wnt1)*. Overexpression of *miR-22* and *miR-34a* inhibits Wnt signaling, which leads to increased lipid accumulation in HepG2 cells.[11]

Fibroblast growth factor 21 (FGF21) is a master metabolic regulator that has a remarkable ability to reverse diabetes and obesity. In addition, FGF21 has regenerative capability and repairs injured tissue. Activation of FGF21 leads to AMPK and ERK1/2 activation. Given the role of FGF21 in metabolism and proliferation, its functions require regulation to avoid metabolism-driven overgrowth, which can be tumorigenic. A recent study has established the relationship between *miR-22* and FGF21 and its receptor fibroblast growth factor receptor 1 (FGFR1) expression.[12] The levels of *miR-22* and FGF21, FGFR1, as well as peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) were inversely correlated in human and mouse fatty livers, suggesting that hepatic *miR-22* acts as a metabolic silencer. Further mechanistic analysis revealed that *miR-22* directly targeted FGFR1. However, *miR-22* decreased FGF21 by reducing the occupancy of transcriptional factors peroxisome proliferator-activated receptor α (PPAR α) and PGC1α to their binding motifs. Thus, *miR-22* can be considered as a metabolic silencer by inhibiting the expression of FGF21 and its receptor.[12] The genes regulated by *miR-22* to reduce metabolism are summarized in Table 1.

3. *MiR-22* in hepatic steatosis and fibrosis

In consistency with the negative role of *miR-22* in regulating hepatic lipid metabolism, *miR-22* is increased in various drug-induced steatosis including drugs like valproate, doxycycline, cyclosporin A, and tamoxifen. M*iR-22* is a potential biomarker for drug-induced steatosis and can be used to predict the effect of a drug on steatosis development.[13] Hepatic *miR-22* overexpression also enhances diet and alcohol-induced steatosis. In

contrast, reducing *miR-22* level up-regulates hepatic FGF21 and FGFR1, leading to AMPK and ERK1/2 activation, which effectively improve alcoholic steatosis in mouse models.[12]

A combination of serum *miR-22* and *miR-210*, which distinguish F0 fibrosis from any fibrosis, can be noninvasive diagnostic biomarkers to detect the presence of liver fibrosis in children with cystic fibrosis.[14]

Furthermore, *miR-22* levels are inversely correlated with the bone morphogenic protein 7 (BMP7) levels in human livers. BMP7 inhibits the progress of liver cirrhosis by inhibiting the expression of transforming growth factor beta 1 (TGF-β1), blocking the nuclear accumulation of SMAD family member 2/3 (Smad2/3), or increasing the level of Gremlin protein secreted by fibroblasts.[15–17] In a carbon tetrachloride-induced cirrhosis mouse models, adeno-associated viruses carrying antisense of *miR-22* significantly attenuated the levels of liver fibrosis, portal hypertension, as well as sodium retention caused, possibly by upregulation of BMP7. Thus, increased *miR-22* promotes liver cirrhosis through directly targeting *BMP7*.[18] In consistency, microarray screening study showed that "mmu_circ_34116/miR-*22*-3P/BMP7" signal axis might be involved in the activation of hepatic stellated cells. Furthermore, transfection experiment validated that the expression of alpha-smooth muscle actin (α-SMA) is significantly elevated because of inhibitory expression of mmu_circ_34,116.[19]

However, *miR-22* inhibits galectin-1 and 9, which are implicated in the development of hepatic fibrosis.[20,21] Down-regulation of galectin-1 can improve liver fibrosis by reducing α-SMA, desmin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin.[22] Serum galectin-9 levels are positive correlation with liver fibrosis.[23] Thus, the role of *miR-22* in liver diseases can be complicated. Whether *miR-22* via reducing galectins can treat hepatic fibrosis remains to be studied.

4. *MiR-22* and viral hepatitis

Serum *miR-22* and *miR-1275* are up-regulated in hepatitis B virus (HBV) patients. The level of those miRNAs are positively correlated with the serum γ-glutamyl transpeptidase levels.[24] In consistency, serum level of *miR-22* and *miR-122* are increased in chronic HBV patients.[25,26] Additionally, their expression levels are positively associated with hepatitis B surface antigen (HBsAg) levels and ALT levels. Similarly, elevated circulating *miR-22* is found in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) patients and is involved in the etiology of liver injury in HIV patients.[27] Further, elevated circulating *miR-22* and *miR-122* indicates viral replication and liver injury in HBV patients.[26]

5. Long non-coding RNA (lncRNA) MIR22HG as a tumor suppressor for hepatocellular carcinoma (HCC)

Based on genome-wide lncRNA expression profiles in HCC tissues and paired adjacent non-tumor tissues, lncRNA NR_028502.1 located in 17p13.3, a chromosomal region that is frequently deleted or hypermethylated in liver cancer, is down-regulated in HCC.[28,29] NR_028502.1 is annotated as the human *miR-22* host gene (*MIR22HG*).[30] Moreover,

reduced *MIR22HG* is related to tumor progression and poor prognosis of patients with HCC. *MIR22HG* overexpression inhibits proliferation, invasion, and metastasis in HCC cells. In part, LncRNA *MIR22HG* acts a tumor suppressor for HCC through deriving *miR-22–3p* to target high mobility group box 1 (*HMGB1*), thereby inactivating HMGB1 downstream pathways.[30]

6. *MiR-22* as a tumor suppressor

MiR-22 expression levels were analyzed in different types of cancer using information available from the TCGA Data Portal. The studies that have normal specimen number greater than 15 were included in the analysis. The data showed that in comparison with normal specimens, *miR-22* levels were differentially expressed based on cancer types. In comparison with normal specimens, its level was reduced in HCC, breast invasive carcinoma, and lung squamous cell carcinoma (Fig. 1A and B).

We further analyzed the relationships between *miR*-22 levels and HCC clinical features. The data showed that the level of *miR*-22 was inversely associated with the depth of HCC invasion. T3 and T4 cancers had lower *miR-22* level compared with T1 and T2 (Fig. 2A and E). In addition, HCC patients at stage III or IV had lower *miR*-22 level than those at stages I and II (Fig. 2B and E). Furthermore, *miR*-22 expression level was positively correlated with overall survival and disease-free survival (Fig. 2C–E). Thus, *miR-22* can be considered as tumor suppressor for HCC.

It has been shown that low expression of *miR-22* is associated with poor prognosis in hepatoma patients.[31] In addition, reduced hepatic or serum *miR-22* is shown in HBV-associated HCC patients. However, no significant difference of serum *miR-22* levels was found between benign liver disease and non-HBV-related HCC patients. In consistency with our data analysis, *miR-22* levels were negatively correlated with tumor size, lymph node metastasis, TNM stage, pathological type, differentiation grade, liver cirrhosis, serum alpha-fetoprotein (AFP) and HBV DNA copy number.[32] Moreover, another study also shows that serum *miR-22* and *miR-199a-3p* in combined with AFP have a high accuracy in early detection of HCC patients with chronic hepatitis C (Table 2).[33]

7. The mechanism by which *miR-22* acts as a tumor suppressor

Several mechanisms by which *miR-22* acts as a liver cancer suppressor have been uncovered. *miR-22* inhibits the development of HCC through directly targeting LncRNA *NEAT1* and AKT serine/threonine kinase 2 (*AKT2*), which are overexpressed in human HCC specimens in comparison with adjacent normal tissue.[34] Both *NEAT1* and *AKT2* are implicated in the development of HCC by increasing proliferation and invasion while inhibiting apoptosis in HCC cells.[34–36]

MiR-22 can also directly target the heterogeneous nuclear ribonucleoprotein A1 (*HNRNPA1*), a potential diagnostic marker for HBV-related HCC.[37] As an oncogene, *HNRNPA1* promotes HBV-related HCC via the EGFR signaling pathway. Additionally, *HNRNPA1* is negatively correlated with the overall survival of HCC patients.

MiR-22 is reduced in folate deficiency-conditioned HCC cell lines including SK-Hep1 and Mahlavu.[38] *MiR-22* overexpression reduces the number of spheres in both liver cancer Sk-Hep1 and Mahlavu (MDA-MB-453) cells, and the opposite is observed by inhibiting *miR-22*. It has been shown that reduced *miR-22* causes folate deficiency-induced cancer stem-like phenotypes via increasing histone deacetylase 4 (*HDAC4*), zinc finger E-box binding homeobox 2 (*ZEB2*), and octamer-binding transcription factor 4 (*OCT4*), but decreasing paired related homeobox 1 (*PRRX1)*.[38]

MiR-22 also silences galectin-1 and 9, which specifically bind to β-galactoside sugars. Galectin-1 is overexpressed in HCC and promotes HCC progression.[39–41] The expression of *miR-22* is negatively correlated with the expression of *galectin-1*. The expression of *galectin-1* is increased in hepatic stellate cells (HSCs) isolated from HCC tissues. *MiR-22* inhibits the HSC-induced T cell apoptosis and cytokine production promoted by HSC-derived galectin-1 in HCC.[20] Moreover, elevated galectin-1 and low CD3 expression levels is associated with poor prognosis in HCC patients. Further, *Galectin-9* is increased while *miR-22* is decreased in human liver cancer tissues and cell lines. *MiR-22* inhibits lymphocyte apoptosis and tumor cell proliferation in HCC cells via silencing *galectin-9*.[21]

MiR-22 can directly target cell cycle gene expression.[42] Cyclin A2 (*CCNA2*) is a direct *miR-22* target gene in both liver and colon cancer cells.[42] *MiR-22* overexpression as well as chemicals that induce *miR-22* expression can reduce CCNA2 protein and increase the number of G0/G1 in human liver cancer Huh7 and colon cancer HCT116 cells.[42]

Silencing multiple protein deacetylases is another mechanism by which *miR*-22 has anti-cancer effects. *HDAC1* is a novel *miR-22* target recently uncovered by our group using colon cancer cells.[43] In a *miR-22*-dependent manner, histone deacetylase (HDAC) inhibitors reduce HDAC1, HDAC4, and sirtuin 1 (SIRT1), which are highly expressed in the liver and colon cancer specimens. Upon *miR-22* induction, reduced HDAC1, HDAC4, and SIRT1 occupied the transcriptional regulatory region of the retinoic acid receptor beta (*RARβ*) and nuclear receptor subfamily 4 group A member 1 (*NUR77*) genes leads to increased H3K9 acetylation of the *RARβ* and *NUR77* genes. Therefore, *miR-22*-reduced protein deacetylases simultaneously induce NUR77 and RARβ expression, as well as, their nuclear export converting their transcriptional effect into apoptotic effect.[43]

Nuclear factor κB (NF-κB) regulates many biological processes including liver tumorigenesis.[44] *MiR-22* inhibits NF-κB activity through targeting NF-κB coactivator, namely nuclear receptor coactivator 1 (*NCOA1*).[44] The mechanisms by which *miR-22* functions as a HCC suppressor is summarized in Table 3 and Fig. 3.

It is interesting to note that the potential tumor-promoting effect of *miR*-22 has also been revealed in an animal model. *miR-22* inhibits the expression of methionine adenosyltransferase 1A (*Mat1a*) and methylenetetrahydrofolate reductase (*Mthfr)* in early preneoplastic livers of rats treated by 2-acetylaminofluorene. The reduced expression of *Mat1a* and *Mthfr* genes by *miR-22* and *miR-29b* is a main driver to promote liver carcinogenesis in the studied model.[45]

8. The mechanisms by which the expression of *miR-22* is regulated

Knockout hepatic nuclear respiratory factor 1 (*Nrf1α*) causes oncogenic activation of NF-E2-related factor 2 (*Nrf2*) and leads to the development of NASH and hepatoma. Thus, Nrf1α functions as a dominant tumor suppressor. It has been shown that both Nrf1α and Nrf2 regulate *miR-22* expression via binding to the antioxidant response element (ARE) site of the *miR-22* promoter.[46]

There are several chemicals that can induce the expression of *miR-22* including catalpol, an iridoid glucoside. Catalpol induces *miR-22* and reduces cell proliferation, invasion, and migration. Catalpol also increases apoptotic rates and G0/G1 phase of Huh7 and HCLMM2 cells. Catalpol exerts anti-tumor effects through up-regulating *miR-22–3p*, which reduces the metastasis associated 1 family member 3 (*MTA3*) expression by directly targeting *MTA3*.[47]

MiR-22 is induced in human liver cancer Huh7 cells treated with sodium butyrate.[42,48] Sodium butyrate treatment or forced *miR-22* overexpression increases the ROS production and reduces SIRT1 expression. Down-regulation of *miR-22* counteracts the effects of butyrate in Huh7cells including the induction of apoptosis via ROS production, cytochrome c release, and activation of caspase-3. Furthermore, anti-*miR-22* also reverses the inhibition of cell growth and proliferation mediated by sodium butyrate.[48]

In addition to butyrate, other short-chain fatty acids (SCFAs) that have histone deacetylase (HDAC) inhibitory property such as propionate and valerate as well as synthetic HDAC inhibitor suberanilohydroxamic acid (SAHA) can also induce *miR-22* as demonstrated using colon cancer cells.[43] In contrast, SCFAs that lack HDAC inhibitory effect such as formate and acetate do not have an effect in inducing *miR-22*.[43] Additionally, retinoic acid (RA), which is produced by butyrate-induced aldehyde dehydrogenase 1 family member A1(ALDH1A1), also induces *miR-22*. Furthermore, when HDAC inhibitors are used in combination with RA, the induction of *miR-22* reaches to a higher level than a single chemical treatment. Such induction is mediated via retinoic acid receptor β (RARβ) binding to a direct repeat 5 (DR5) motif found in the regulatory region of the *miR*-22.[43]

Bile acids via its receptor farnesoid X receptor (FXR) induces *miR*-22 by direct binding to an invert repeat 1 (*IR-1*) motif located in the regulatory region of the *miR-22*.[42] Both endogenous FXR ligand chenodeoxycholic acid and synthetic FXR ligand GW4064 increase *miR-22* in Huh7 liver and HCT116 colon cells.[42] In addition, semi-synthetic bile acid, obeticholic acid, which is in clinical trials to treat NASH, also increases *miR-22* expression in Huh7 liver cells.[12] Furthermore, by inducing FGF21 signaling, *miR-22* inhibitors can improve the effect of obeticholic acid in improving insulin sensitivity as demonstrated in Western diet-induced obese mice.[12]

The liver is a testosterone-responsive organ. Testosterone regulates liver metabolism, inhibits hepatic immune responses and even promotes liver carcinogenesis.[49–54] Testosterone treatment of female mice induces hepatic *miR-22*, *miR-690*, *miR-122*, *let-7A*, *miR-30D*, and *let-7D*. An androgen response element (ARE) has been found in the *miR-122* promoter, but not in the other five induced miRNAs. Therefore, the mechanism by which testosterone

induces *miR-22* remains to be uncovered. The induction of *miR-22* leads to reduced expression of estrogen receptor α and aromatase, thus resulting in estrogen signal inhibition.[55]

The chemicals that have anti-cancer effects and can increase the expression level of *miR-22* are summarized in Table 4 and Fig. 3. It is interesting to note that most of those *miR-22* inducers have metabolic stimulating effects, and yet *miR-22* functions as a metabolic silencer.

9. Conclusion

The level of *miR-22* rises in hepatic steatosis and declines in liver cancer. Thus, *miR-22* inhibition can treat NAFLD, and yet *miR-22* inducers or mimics can be useful treating liver cancer. Indeed, the cancer treatment effects of *miR-22* inducers includes catalpol, butyrate, retinoic acid, and HDAC inhibitors have been revealed.

Metabolism driven by FGF21 leads to AMPK and ERK1/2 activation thereby supporting growth and cell proliferation. Surprisingly, metabolism enhancers such as bile acids, testosterone, and retinoic acid induce the expression of *miR-22*, which silences FGF21 and its receptor. The simultaneous induction of *miR-22* as well as FGF21 signaling likely maintain FGF21 homeostasis and restrict persistent ERK1/2 activation. In other words, concomitant induction of FGF21 and *miR-22* can be a way to maintain FGF21 homeostasis and thus insulin sensitivity. However, the reduction of *miR-22* may improve the efficacy of AMPK activators by increasing hepatic FGF21.

The expression level of *miR-22* changes in a dynamic way as liver disease progresses. To use *miR-22* as a drug target, the status of *miR-22* needs to be monitored. *miR-22* is also ubiquitously expressed and it has many other important biological functions such as immunity regulation.[56,57] In other types of cancer, *miR-22* may function differently. Targeted delivery of *miR-22* inducer or silencer should be considered to avoid unwanted effects.

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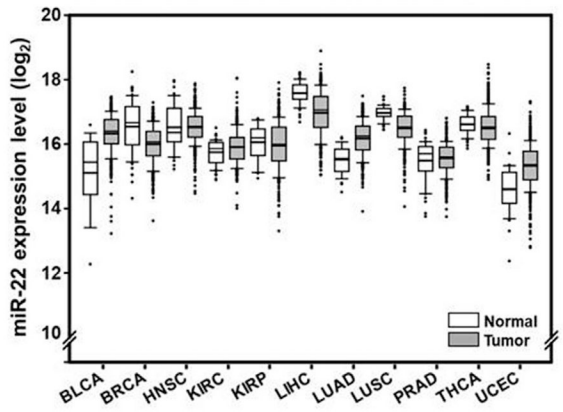
A

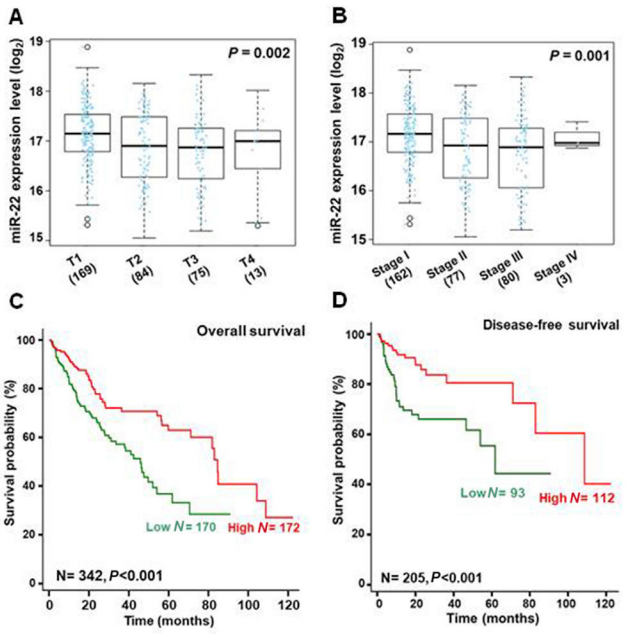


B

Cancer type		Sample size		Mean ± SD		P -value
Acronym	Full name	Normal	Tumor	Normal	Tumor	
BLCA	Bladder Carcinoma	19	409	15.1±1.2	16.3±0.6	**< 0.001**
BRCA	Breast Invasive Carcinoma	74	501	16.5±0.8	16.0±0.6	**< 0.001**
HNSC	Head-Neck Squamous Cell Carcinoma	44	482	16.5±0.7	16.5±0.5	0.971
KIRC	Kidney Renal Clear Cell Carcinoma	71	324	15.7±0.4	15.9±0.5	**0.019**
KIRP	Kidney Renal Papillary Cell Carcinoma	34	324	16.1±0.6	15.9±0.8	0.432
LIHC	Liver Hepatocellular Carcinoma	53	370	17.6±0.3	17.0±0.7	**< 0.001**
LUAD	Lung Adenocarcinoma	39	450	15.5±0.4	16.2±0.5	**< 0.001**
LUSC	Lung Squamous Cell Carcinoma	46	338	17.0±0.2	16.5±0.5	**< 0.001**
PRAD	Prostate Adenocarcinoma	50	479	15.5±0.6	15.6±0.5	0.315
THCA	Thyroid Cancer	57	501	16.6±0.3	16.5±0.5	0.204
UCEC	Uterine Corpus Endometrial Carcinoma	32	409	14.6±0.8	15.3±0.7	**< 0.001**

Fig. 1. *MiR*-22 expression level in different cancers.

The *miR*-22 expression level (log_2) was analyzed using TCGA Data Portal and data are shown as box plot (white box, normal specimens; gravy box: cancer specimens).



E

		MiR-22 expression		
	Total (n)	Low (%)	High (%)	*P*-value
Gender				
M	237	107 (45.1)	130 (54.9)	0.819
F	112	49 (43.8)	63 (56.2)	
Age (years)				
<65	207	112 (54.1)	95 (45.9)	0.059
≥65	134	58 (43.3)	76 (56.7)	
Depth of tumor invasion				
T1+T2	253	117 (46.2)	136 (53.8)	0.024
T3+T4	88	53 (60.2)	35 (39.8)	
Lymph node metastasis				
No	241	124 (51.5)	117 (48.5)	1.000*
Yes	3	2 (66.7)	1 (33.3)	
Distant metastasis				
No	247	125 (50.6)	122 (49.9)	1.000*
Yes	3	2 (66.7)	1 (33.3)	
Stage (UICC)				
I + II	239	108 (45.2)	131 (54.8)	0.018
III + IV	83	50 (60.2)	33 (39.8)	
Status				
Live	222	98 (44.1)	124 (55.9)	0.005
Dead	120	72 (60.0)	48 (40.0)	

*Fisher exact test

Fig. 2. The associations between *miR*-22 levels and HCC clinical features.

The correlation between *miR*-22 expression level (log_2) and **(A)** the depth of tumor invasion and **(B)** tumor stages. Kaplan-Meier curves showed the relationships between *miR-22* levels and **(C)** overall survival and **(D)** disease-free survival. *P*-values were calculated by the log-rank test. Clinical features and *P* values are summarized in **(E)**.

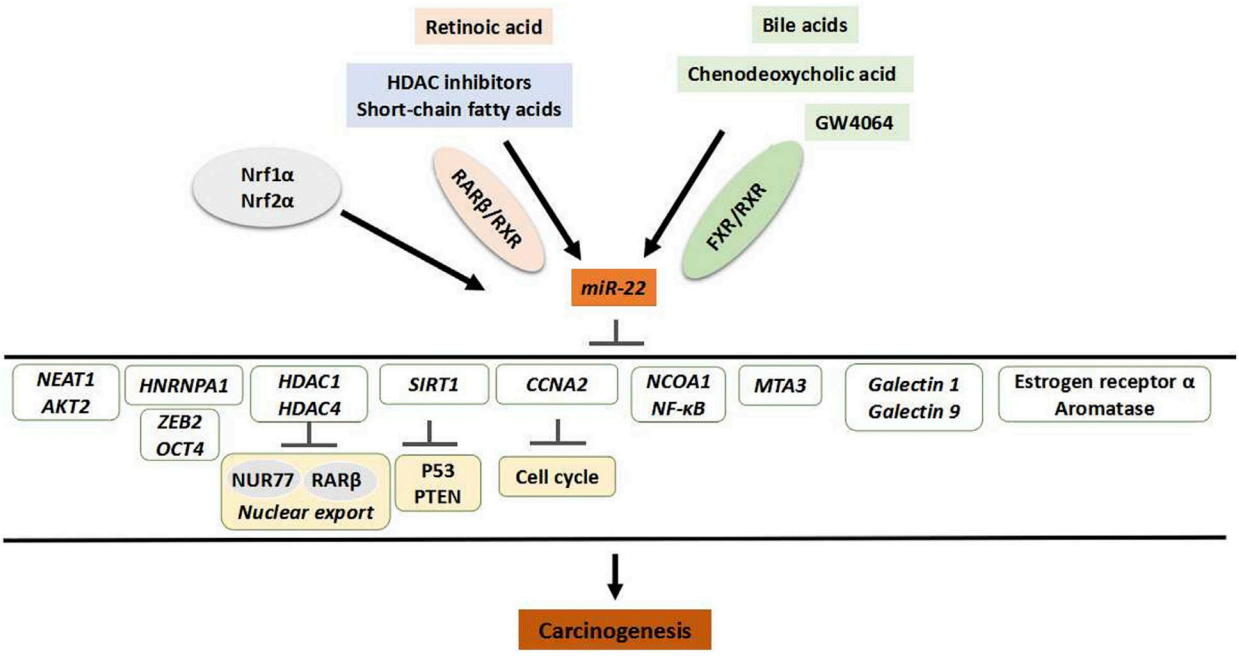


Fig. 3. The regulation and function of *miR-22*.

MiR-22 can be induced by ligands for nuclear receptors such as retinoic acid, bile acids, as well as chemicals that have HDAC inhibitory property. Transcription factor Nrf1α and Nuf2α also induce *miR-22*. All those chemicals and transcriptional factors have known anti-cancer effects. Abbreviations: *miR-22*,microRNA 22; HDAC, histone deacetylase; Nrf1α, nuclear respiratory factor 1; Nrf2α, NF-E2-related factor 2a; RARβ, retinoic acid receptor beta; FXR, farnesoid X receptor; HNRNPA1, heterogeneous nuclear ribonucleoprotein A1; ZEB2, Zinc finger E-box binding homeobox 2; OCT4, octamer-binding transcription factor 4; NUR77, nuclear receptor subfamily 4 group A member 1; SIRT1, sirtuin 1; PTEN, phosphatase and tensin homolog; CCNA2, cyclin A2; NCOA1, nuclear receptor coactivator 1; NF-κB, nuclear factor kappa B; MTA3, metastasis associated 1 family member 3.

Table 1

MiR-22 as a metabolic silencer.

Regulated genes	Function	Refs.
Srebp-1, Ccl2, Il-6, Ifng	Lack of *miR-22* prohibits fat mass formation and dyslipidemia caused by a high-fat diet	10
Ep300, Tcf7	*MiR-22* inhibits Wnt signaling leading to increased lipid accumulation in HepG2 cells	11
FGFR1, FGF21	Increased hepatic *miR-22* and reduced FGF21 are found in hepatic steatosis	12

Abbreviations: miR-22,microRNA 22; Srebp-1, sterol regulatory element binding protein-1; Ccl2, CC motif chemokine ligand 2; Il-6, interleukin 6; Ifng, interferon gamma; Ep300, E1A binding protein p300; Tcf7, transcription factor 7; FGFR1, fibroblast growth factor receptor 1; FGF21, fibroblast growth factor 21.

Table 2

MiR-22 as a liver cancer diagnostic marker.

Diagnostic indicator	Disease	Refs.
Reduced expression of *miR-22*	Poor prognosis in hepatoma in patients	31
Reduced hepatic or serum *miR-22*	HBV-associated HCC patients.	32
Serum *miR-22* and *miR-199a-3p* in combination with AFP	Early phase of HCC in patients with chronic HCV	33

Abbreviations: *miR*, microRNA; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; AFP, alpha-fetoprotein.

Table 3

The mechanisms by which *miR-22* acts as a liver cancer suppressor.

Cancer models	Target genes	Function of the target genes	Refs.
Human HCC	*NEAT1, AKT2*	Promote proliferation and invasion, inhibit Apoptosis	34–36
HBV-related HCC	*HNRNPA1*	Regulates EGFR signaling	37
Folate deficiency-conditioned HCC cells	*HDAC4, ZEB2, OCT4, PRRX1*	Regulate gene expression	38
Human HCC	*Galectin-1, Galectin-9*	Promote T cell apoptosis and cytokine production	39–41
Huh7 and HCT116cells	*CCNA2*	Regulates cell cycle	42
HCT116 and DLD-1 cell	*HDAC1, HDAC4, SIRTJ, NUR77, RARb*	Epigenetic and transcriptional regulation leading to apoptosis of cancer cell	43
Huh7 cell	*NCOA1, NF-kB*	Transcriptional regulation	44

Abbreviations: *miR-22*, microRNA 22; HCC, hepatocellular carcinoma; *AKT2*, AKT serine/threonine kinase 2; HBV, hepatitis B virus; HNRNPA1, heterogeneous nuclear Ribonucleoprotein A1; HDAC, histone deacetylase; ZEB2, Zinc finger E-box binding homeobox 2; OCT4, octamer-binding transcription factor 4; PRRX1, decreased paired related homeobox 1; CCNA2, cyclin A2; SIRT1, sirtuin 1; NUR77, nuclear receptor subfamily 4 group A member 1; RARβ, retinoic acid receptor beta; NCOA1, nuclear receptor coactivator 1; NF-κB, nuclear factor kappa B.

Table 4

MiR-22 inducers.

***MiR-22* inducers**	**Refs.**
Catalpol	47
Bile acids, Chenodeoxycholic acid, GW4064, Obeticholic acid	12,42
Retinoic acid	43
HDAC inhibitors: butyrate, propionate, valerate, suberanilohydroxamic acid	42,43,48
Testosterone	55

Abbreviations: *miR-22*, microRNA 22; HDAC, histone deacetylase.