Advances in the study of the mechanism of action of miR-22 in liver lesions (Review)

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Abstract. Globally, nearly 2 million deaths annually are attributed to the development of liver diseases, with liver cancer and cirrhosis being particularly prominent, which makes liver disease a significant global health concern. Cirrhosis is closely linked to the evolution of hepatitis, hepatic fibrosis and fatty liver. However, most liver diseases have an insidious onset, are challenging to treat and the prognosis and efficacy of current therapies are unsatisfactory, which can result in irreversible functional damage to the liver. Therefore, there is an urgent need to explore the molecular mechanisms underlying liver disease and identify new biomarkers and therapeutic targets. In previous years, microRNAs (miRs), a class of short non-coding RNAs comprising 17-25 nucleotides, have attracted attention for their roles in various types of liver diseases. Among them, miR-22 serves a unique role in mediating multiple pathway mechanisms and epigenetic modifications and can act both as an inhibitor of liver cancer and a metabolic blocker. Given its close association with the liver, several studies have reported that the differential expression of miR-22 regulates the metabolic process of liver cancer and is involved in the evolution of hepatic fibrosis and steatohepatitis, making it a potential target for early diagnosis and treatment. The present manuscript aimed to comprehensively review the key role of miR-22 in the evolution of liver diseases and offer valuable references and guidance for subsequent studies by identifying its specific mechanism of action and future development prospects.

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Abbreviations: HCC, hepatocellular carcinoma; miRNA, microRNA; UTR, untranslated region; HDAC4, histone deacetylase 4; HMGB1, high mobility group box 1; CBL, casitas B-lineage lymphoma; lncRNA, long non-coding RNA; SPRY2, sprouty2; DSCR8, down syndrome critical region 8; ARPC5, actin-related protein 2/3 complex subunit 5; MKLN1-AS, muskelin 1 antisense RNA; ETS1, ETS proto-oncogene 1; NCK1-AS1, NCK1 antisense RNA 1; YARS, tyrosyl-tRNA synthetase; MIAT, myocardial infarction-associated transcript; SIRT1, sirtuin 1; ROS, reactive oxygen species; AFP, alpha fetoprotein; CHB, chronic hepatitis B; MTA3, metastasis associated 1 family member 3; CCNA2, cyclin A2; FXR, farnesoid X receptor; JARID2, jumonji AT rich interacting domain containing 2; HIF1α, hypoxia-inducible factor 1 alpha; Gal-9, galectin-9; UBE4B, ubiquitin ligase E4B; FGD5-AS1, FGD5 antisense RNA 1; LINC00858, long intergenic non-protein coding RNA 858; SNHG16, small nucleolar RNA host gene 16; HBV, hepatitis B virus; HCV, hepatitis C virus; HNRNPA1, heterogeneous nuclear ribonucleoprotein A 1; HBx, HBV-encoded X; YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; TRERNA1, translation regulatory lncRNA 1; ERα, estrogen receptor α; NRAS, NRAS proto-oncogene; EZH2, enhancer of zeste homolog 2; NASH, nonalcoholic steatohepatitis; AFLD, alcoholic fatty liver disease; ALD, alcoholic liver disease; NAFLD, non-AFLD; FGF21, fibroblast growth factor 21; FGFR1, FGF21 receptor; PGC1α, hepatic PPAR-activated receptor-c coactivator-1α; PPARα, peroxisome proliferator-activated receptor α; HFD, high-fat diet; miR-22KO, miR-22 knock-out; T2DM, type 2 diabetes mellitus; T2, 3,5-diiodine-L-thyronine; TCF7, transcription factor 7; HSC, hepatic stellate cell; ECM, extracellular matrix; Neat1, nuclear paraspeckle assembly transcript 1; ceRNA, competing endogenous RNA; SIL, silymarin; DILI, drug-induced liver injury

Key words: microRNA-22, hepatocellular carcinoma, alcoholic fatty liver disease, non-alcoholic fatty liver disease, liver fibrosis, viral hepatitis

Contents

1. Introduction

Globally, the burden of liver diseases results in 2 million deaths annually (1). These diseases include liver cancer, viral liver disease, cirrhosis, non-alcoholic fatty liver disease (NAFLD), AFLD and drug-induced liver injury (DILI).

Hepatocellular carcinoma (HCC), which accounts for a significant proportion of liver diseases, is the sixth most common and the fourth most lethal type of all cancers worldwide (2). Despite advancements in modern medicine, such as surgical resection, local ablation, percutaneous intervention and liver transplantation, the highly invasive and metastatic nature of HCC complicates complete surgical resection (3,4). Simultaneously, the current first-line chemotherapeutic drug, sorafenib, which is used for the treatment of HCC, encounters challenges related to drug resistance (5), which significantly contributes to recurrence following treatment (6,7). Cirrhosis, a prevalent chronic liver disease that can lead to death, can be caused by various factors, such as viral hepatitis and fatty liver disease. Inflammatory stimulation causes the liver to develop fibrotic lesions, which eventually deform and harden. Viral infections, particularly hepatitis B and C, can result in severe liver damage, cirrhosis and liver cancer. Hepatitis B virus (HBV) and HCV infections are responsible for 75% of liver cancer cases. Improvements in socioeconomic conditions and lifestyle changes have increased the rates of obesity and alcohol consumption, which contributes to the rising prevalence of fatty liver disease (8,9). Fatty liver disease is characterised into AFLD and NAFLD and is one of the most prevalent liver diseases worldwide, which is accompanied by complex clinical symptoms, such as certain metabolic-related syndromes (10). Therefore, effective biomarkers, therapeutic targets, and screening strategies are necessary for the early diagnosis, treatment and prognosis of liver diseases, including cancer.

In previous years, the discovery of effective biomarkers has significantly contributed to the early diagnosis and treatment of liver diseases (11). Among these biomarkers, microRNA (miRNA/miR)-22, which has been proved by numerous studies to be associated with liver cancer and affect the occurrence and development of liver cancer, has attracted considerable attention (12-14). miR-22 belongs to the miRNA class, comprising short non-coding RNAs of 17-25 nucleotides that can serve an important regulatory role in tumour development (15). They inhibit target gene expression by targeting the 3' untranslated region (3'-UTR) of genes (16). miR-22 exists in two functional forms, miR-22-3p (or miR-22) and miR-22-5p (or miR-22β). miR-22-3p serves as a functional guide strand and regulates its target by being complementary, whereas miR-22-5p is generally regarded as a transient strand that is easily degraded (17). miR-22-3p, as a tumour suppressor, inhibits the development of various types of cancers, including breast (18), non-small-cell lung (19), gastric (20) and colorectal cancers (21). The role of miR-22 in the liver has attracted research attention. A clinical study reported lower miR-22 expression levels in patients with HCC compared with healthy controls, which suggests a potential role as a tumour suppressor in HCC (22). Furthermore, miR-22 serves a unique role in fatty liver disease and liver fibrosis (23,24). miR-22 can also reflect abnormal glucose-lipid and alcohol metabolism in the liver, which may contribute to future research on the detection and prevention of liver disease. In addition, miR-22 has been linked to liver lesions, such as liver cancer, fatty liver disease and liver fibrosis (25-27). miR-22-3p has been shown to inhibit the development of various liver diseases, while miR-22-5p acts as an early diagnostic biomarker for acute myocardial infarction (28,29).

However, reports on the role of miR-22 in liver diseases are currently limited and the underlying molecular mechanism of action has not been comprehensively elucidated. Herein, the mechanism of action of miR-22 in the progression of liver lesions was comprehensively and systematically reviewed in the present manuscript and covered numerous stages of liver lesion development such as fatty liver, liver fibrosis and liver cancer.

2. miR-22 and liver cancer

MIR22HG, the host gene of human miR-22, belongs to the long non-coding RNA (lncRNA) family located on chromosome 17p13.3 (30), and is involved in regulating bio-signalling in multiple types of cancer cells; therefore, MIR22HG can also act as a tumour suppressor gene that inhibits the development of various types of cancer (31-34). Conversely, MIR22HG can act as a tumour promoter in oesophageal adenocarcinoma and glioblastoma (34), which highlights its complex biological functions. With recent advancements in liver cancer research, the role of miR-22 as an inhibitor of liver cancer has received attention (35). In the following sections, the molecular mechanisms of HCC development, early diagnosis and prognosis will be reviewed to provide insights into the role and effect of MIR22HG and related molecules in HCC.

Mechanisms by which miR-22 regulates HCC development. As a tumour suppressor, miR-22 regulates the expression of tumour-related factors via multiple pathways, which can inhibit liver cancer occurrence and development (36-38). Conversely, miR-22 deletion promotes the occurrence and development of tumours *in vivo* (39). The expression levels of miR-22 are significantly downregulated in HCC and show a gradual decrease with the continuous progression of cancer stages (13,39,40). Multiple mechanisms associated with miR-22 have been reported to be involved in the occurrence and development of liver cancer (40,41).

miR-22 inhibits the proliferation, migration and invasion of HCC cells and promotes apoptosis. Zhang *et al* (35) reported that miR-22 overexpression in HCC tissues significantly reduced histone deacetylase 4 (HDAC4) expression, thereby inhibiting the proliferation of Hep3B and SMMC7721 HCC cells. This inhibitory effect was confirmed both *in vivo* and *in vitro*. The aforementioned study also reported that miR-22-3p and MIR22HG were co-expressed and exhibited a synergistic function. In HCC, MIR22HG functions as a competitive endogenous RNA (ceRNA), which regulates miRNA-10a-5p/nuclear receptor corepressor 2 to inhibit the Wnt/β-catenin signalling pathway, thereby inhibiting the growth, invasion and migration of HCC cells (42). Simultaneously, miR-22-3p derived from MIR22HG interacts with human antigen R to reduce β-catenin expression and target high mobility group box 1 (HMGB1) to inhibit HCC cell migration and invasion (43). Similarly, Luo *et al* (44) reported that miR-22 overexpression reduced CD147 expression, inhibiting HCC migration and invasion. Casitas B-lineage lymphoma (CBL) is a direct target of miR-22-3p and the ubiquitin protein ligase (E3) of sprouty2

(SPRY2). By inhibiting CBL expression, miR-22-3p can reduce SPRY2 ubiquitination and indirectly upregulate SPRY2 expression, thereby inhibiting ERK signal transduction. This hindrance of the MAPK/ERK signalling process inhibits the epithelial-mesenchymal transition, migration and invasion of HCC cells, which contributes to cancer inhibition (17,45).

However, the function of miR-22 in liver cancer is negatively regulated by lncRNAs. lncRNAs comprise >200 nucleotides and are the most common type of non-protein-coding transcripts (46). lncRNAs can act as ceRNA, sequestering specific miRNAs from their target genes, which reduces the abundance of their target miRNAs and inhibits their stability and function. Multiple ceRNAs have a sponging effect on miR-22 and influence its regulation of proliferation and apoptosis in liver tumour cells, weakening or reversing the tumour-inhibitory effect of miR-22 (47). Huang *et al* (37) reported that lncRNA DSCR8, a ceRNA of miR-22-3p, prevented miR-22-3p from binding to the target actin-related protein 2/3 complex subunit 5 (ARPC5) through sponging, thereby failing to reduce the inhibitory effect of ARPC5 on tumour cell apoptosis, which ultimately promoted liver cancer progression. Additionally, muskelin 1 antisense RNA (MKLN1-AS) contributes to the growth and development of HCC cells (48). Considering these findings, Pan *et al* (49) conducted RNA immunoprecipitation (RIP) and reported that MKLN1-AS and miR-22-3p were enriched in the anti-argonaute 2 group compared with those in the anti-IgG group. miR-22-3p overexpression can directly downregulate ETS proto-oncogene 1 (ETS1) in HuH7 and LM3 cells to regulate protein levels related to cell proliferation, apoptosis and migration. MKLN1-AS sponges miR-22-3p, indirectly upregulates ETS1 expression, induces cell growth, angiogenesis, migration and invasion and promotes the occurrence and development of HCC. ETS1 also regulates MKLN1-AS expression. ETS1 knockdown has been reported to reduce the expression level of MKLN1-AS, whereas its overexpression increases MKLN1-AS levels. ETS1 was reported to bind to the MKLN1-AS promoter site 3.

The NCK adaptor protein 1 antisense RNA 1 (NCK1-AS1) gene is typically found in the cytoplasm and serves a role in processes that occur after genetic transcription (50). When NCK1-AS1 is suppressed, the expression levels of miR-22-3p are elevated, which increases the number of cells undergoing apoptosis; conversely, when miR-22-3p is silenced, the increase in the number of apoptotic cells caused by NCK1-AS1 knockdown is reversed and the proliferation, migration and invasion abilities of certain cells are also affected (51). This suggests an inverse relationship between NCK1-AS1 and miR-22-3p in liver cancer tissues (52). The presence of NCK1-AS1 in HCC tissues is linked to increased levels of tyrosyl-tRNA synthetase (YARS) expression, while miR-22-3p decreases the levels of YARS. Therefore, NCK1-AS1 acts as a sponge by binding to miR-22-3p to increase YARS expression levels, thus suppressing tumour cell apoptosis and promoting cell growth and migration. Additionally, suppressing YARS impedes the activation of key members of the PI3K/AKT pathway (PI3K, AKT, ERK and mTOR), thereby hindering cell proliferation (53). Therefore, when positively regulating YARS through the miR-22-3p/YARS axis, NCK1-AS1 can activate PI3K/AKT signalling to promote HCC progression. However, silencing NCK1-AS1 or overexpressing miR-22-3p can reverse this process and serve a role in liver cancer inhibition (52).

Zhao *et al* (47) reported that myocardial infarction-associated transcript (MIAT), an aging-related lncRNA involved in HCC, was upregulated in human HCC and served a role in tumour promotion. The expression level of MIAT decreases during cell aging, whereas overexpression of MIAT can inhibit cell aging and hinder tumour cell apoptosis. MIAT acts as a ceRNA by binding specifically to miR-22-3p, inhibiting miR-22 expression and increasing sirtuin 1 (SIRT1) expression, which is a direct target of miR-22. This inhibits tumour suppressor pathways p53/p21 and p16/pRb, which in turn, inhibit the production of senescence-associated secretory phenotype and cell senescence, promoting tumour cell proliferation and inhibiting apoptosis. Overexpression of miR-22-3p significantly decreases SIRT1 levels. Therefore, specific binding of MIAT and miR-22-3p can inhibit the senescence phenotype and promote HCC progression by upregulating SIRT1. Overexpression of miR-22-3p can reverse this phenomenon and promote cell senescence in the human fibroblast cell lines 2BS, IMR-90 and MRC-5. Conversely, miR-22-3p downregulation prevents the progression of cell senescence and improves senescent cells.

miR-22 can not only be inhibited by the above lncRNAs, but also induced by vitamin D3, bile acids and the following exogenous substances. The positive regulation of butyrate on miR-22 can inhibit the expression of SIRT1 and subsequently promote the expression of PTEN and GSK-3, and promote the accumulation of ROS, which not only reduces the expression of phosphorylated (p)-AKT and β-catenin, but also releases cytochrome C to promote cell apoptosis and serve an anti-cancer role (54). Catalpol is an exogenous substance similar to butyrate. After being induced by catalpol, miR-22-3p is negatively regulated by targeting metastasis associated 1 family member 3 (MTA3), thus inhibiting the promotion of MTA3 on the proliferation, migration and invasion of HCC cells (41). In addition, chenodeoxycholic acid can effectively activate the bile acid receptor farnesol X receptor (FXR) in Huh7 and HCT116 cells. FXR binds to the IR1 motif upstream of miR-22 and induces the expression of miR-22 in Huh7 liver cells, thus reducing the expression level of cyclin A2 (CCNA2) mRNA, and the negative correlation of miR-22 and CCNA2 is similarly demonstrated in the clinical data (36). Similarly, miR-22 mimics increased the percentage of Huh7 and HCT116 cells in G0/G1 phase and decreased the percentage of cells in S and G2 phases, which indicated that miR-22 can inhibit the proliferation of tumour cells by interfering with the cell cycle (36). In addition, waltonitone can be used as an upstream regulatory substance to participate in the FXR-miR-22-CCNA2 pathway to inhibit the occurrence and development of liver cancer, and this potent correlation between waltonitone's efficacy and the pathway-mediated inhibition of tumor proliferation has been shown through analysis clinical tissue samples in the Gene Expression Omnibus database (55).

In summary, miR-22 can inhibit cell growth or signal transduction of cancer-promoting pathways by reducing the expression level of proteins such as CD147, HDAC4, CBL and HMGB1 to inhibit the proliferation, migration and invasion of HCC cells. However, the expression levels of miR-22 can be inhibited by various lncRNA such as DSCR8, NCK1-AS1,

MKLN1-AS and MIAT, which prevents miR-22 from exerting its anti-cancer effects. miR-22 can also be induced by FXR, butyrate and catalpol, which can limit the proliferation, migration and invasion of liver cancer cells by affecting the normal progression of the cell cycle. Therefore, miR-22 serves an indispensable role in the regulation of liver cancer growth and has the potential to be used as an effective target for future liver cancer treatment.

Immunomodulatory function of miR-22 in HCC. The occurrence and development of tumours is closely linked to immune regulation. Immune cells and related factors, such as tumour-related macrophages, lymphocytes and mast cells, serve a crucial role in initiating the body's anti-tumour response through direct killing, antigen presentation and immune response activation; immune cells also influence the metabolism of tumor cells (56). This forms the basis for regulating the body's immune system and maintaining the balance of the tumour microenvironment (57). Dysregulation of T cells and their effector lymphocytes can lead to immune escape by tumour cells (58).

miR-22 is involved in the immunomodulatory effects observed in HCC. T helper 17 (Th17) cells, a type of $CD4^+$ helper T cell that produce IL-17, are controlled by the transcription factor retinoic acid receptor-related orphan receptor γt (RORγT), an isomer of the RAR-related orphan receptor C (RORC) (59). Zhang *et al* (40) reported that when injected into the subcutaneous tumours of mice with HCC, miR-22 expression in T cells and tumour cells significantly increased, thereby decreasing tumour size and weight. miR-22 overexpression inhibits tumour growth by promoting the transformation of $CD4^+$ T cells into Th17 cells, while jumonji AT-rich interacting domain containing 2 (JARID2) hinders the production of Th17 cells. miR-22 inhibits JARID2 expression in T cells by directly targeting the JARID2 3'-UTR, which weakens JARID2-mediated inhibition. This aids in maintaining the normal differentiation of Th17 cells and regulating tumour cell apoptosis.

Hypoxic and hypoxia-inducible factor 1 alpha (HIF1α) induces resistance in tumour cells to cytotoxic T cells (60). miR-22 silences HIF1α, which reduces its signal transduction capacities and the resistance of tumour cells and serves a tumour suppressive role. miR-22 also exerts an anti-HCC effect by reducing the recruitment of HIF1α/RORγT/STAT3 to the IL-17 promoter, which inhibits IL-17 signalling in T cells. However, HIF1α reduction can also reduce the binding and expression of RORC and IL-17 and HIF1α/RORγT recruitment to IL-17a. Conversely, miR-22 inhibits the IL-23/IL-6/STAT3 signalling pathway, reduces the recruitment of STAT3 to IL-17a and inhibits the expression level of IL-17. Therefore, treating with miR-22 can reduce the abundance of IL-17-producing T cells, inhibit the IL-17-induced inflammatory response and activate cytotoxic T cells to exert anti-HCC effects (13). Additionally, regulatory T cells (Tregs) have immunosuppressive functions in multiple cancers and miR-22 can inhibit tumour immune evasion by limiting Treg expansion and activating anti-tumour effector cells (61,62). In conclusion, miR-22 serves an important role in the immune regulation of HCC and influences the occurrence and development of HCC by regulating immune processes.

In liver cancer, galectin-9 (Gal-9) induces lymphocyte apoptosis and the immune escape of tumour cells, and Tim-3 is an important inhibitory receptor in the tumour microenvironment; Gal-9 induces apoptosis of HCC cells in the absence of Tim-3 (63,64). A previous study reported the involvement of Gal-9 in tumour immune escape by inducing tumour-specific $Tim3^+$ T cell death (65). Yang *et al* (66) reported that Gal-9 was significantly elevated in human hepatoma cells, particularly in HepG2 cells, when compared with normal hepatocytes. The aforementioned study also used flow cytometry and a WST-1 assay to report that Gal-9 promoted lymphocyte apoptosis and aided tumour cells in evading the immune system. Additionally, HepG2 cells with higher Gal-9 expression levels have higher proliferation capacity than negative control cells. However, overexpression of miR-22 inhibited the expression of Gal-9 and its interaction with Tim-3, thus reducing lymphocyte apoptosis which partly restored the function of effector T cells and regulated the immune response to the tumour, in turn, decreasing tumour cell proliferation and immune evasion. Ubiquitin ligase E4B (UBE4B), a novel E3 protein, belongs to the U-box family of ubiquitin ligases. Shao *et al* (67) constructed a ceRNA network using bioinformatics data analysis and showed that UBE4B was a pro-tumourigenic protein crucial for HCC development. UBE4B acts through the UBE4B-hsa-miR-22-3p-FGD5-AS1/LINC00858/SNHG16 axis to regulate immune processes with a pro-carcinogenic role in HCC development, which leads to poor prognosis and tumour immune infiltration in HCC.

Role of miR-22 in hepatitis virus-associated HCC. Viral hepatitis is primarily caused by viral infections that lead to liver lesions, which is a major contributor to liver disease progression. HBV and HCV infections significantly increase the risk of HCC, and chronic viral infections can lead to liver cirrhosis, which may ultimately progress to HCC (68). Research on miR-22 in hepatitis virus-induced liver disease has focused mainly on the regulation of HCC (69,70). Therefore, the difference between changes in miR-22 expression levels in hepatitis virus-induced liver cancer and other types liver cancer is of interest. Shi and Xu (71) reported that in HBV-associated HCC cells, such as HepG2.2.15, miR-22 expression showed a more significant downward trend compared with that in HepG2 cells, a trend also observed in miR-22 expression in clinical specimens. Furthermore, CDK inhibitor 1A expression was significantly reduced in HCC cells transfected with miR-22 compared with that in control cells, which suggests that miR-22 serves an inhibitory role by interfering with the normal tumour cycle. Additionally, miR-22 transfection significantly inhibits hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg) expression. Although experimental results suggest that miR-22 can strongly inhibit HBV gene expression, the specific mechanism by which this occurs warrants further investigation.

Ke *et al* (70) reported that heterogeneous nuclear ribonucleoprotein A 1 (HNRNPA1) expression was significantly elevated in HBV-positive HCC samples and correlated with a poor prognosis in patients with HCC. HNRNPA1 expression was significantly upregulated, while miR-22 expression was significantly downregulated in HCC cells compared with that in normal hepatocyte cell lines; however, and the difference

between HBV-positive HCC cells and normal cells was more obvious. miR-22 overexpression resulted in suppressed HNRNPA1 expression and EGFR signalling pathway activity. However, HBV-negative HCC cells were not used as a control group for this procedure; hence, the effects of miR-22 on HBV-negative HCC cells warrant further study.

The FOXO3a protein can reduce the invasiveness of HCC cells by blocking the WNT/β-catenin pathway and regulating proteins associated with lymph node metastasis (72,73). Chen *et al* (38) reported that p-FOXO3a can be moved from the nucleus to the cytoplasm by p-AKT, which reduces its activity through ubiquitination and phosphorylation. Conversely, miR-22 can counteract the interference of p-AKT on FOXO3a by inhibiting YWHAZ-mediated AKT phosphorylation. This allows FOXO3a to maintain its tumour-suppressing role in the nucleus.

In addition, using an anti-Ago2 RIP assay, Song *et al* (14) reported that translation regulatory lncRNA 1 (TRERNA1) induced by hepatitis B virus-encoded X (HBx), acts as a sponge for miR-22-3p to regulate NRAS proto-oncogene (NRAS) expression. During tumour formation, TRERNA1 competes with miR-22-3p, thereby elevating NRAS expression levels and HCC cell proliferation by eliminating the NRAS/Raf/MEK/ERK pathway. Conversely, TRERNA1 knock down lowered NRAS expression levels, which were restored following treatment with an miR-22-3p inhibitor. This suggests that in the absence of TRERNA1 sponging, miR-22-3p inhibits the activating effect of NRAS on the NRAS/Raf/MEK/ERK pathway, which ultimately inhibits HCC progression by hindering cell proliferation. Furthermore, the upregulation of TRERNA1 by HBx contributes to sorafenib resistance in HCC cells.

The miR-22/estrogen receptor (ER)α/IL-1α/IL-6 pathway is linked to liver tumours induced by HBV. IL-1α is increased during liver cell death caused by ROS. This increase stimulates Kupffer cells to increase the expression levels of IL-6, which leads to the compensatory growth of damaged liver tissue and the formation of tumours (74,75). In HCC associated with HBV, oestrogen, in combination with ERα, may inhibit IL-6 and IL-1α to protect the liver (76). Additionally, miR-22 can hinder the production of ERα by directly targeting its 3'-UTR region, thereby impeding its influence downstream (76,77); Chronic hepatitis infections, especially with HBV, increases oestrogen production in women, which suppresses the expression of IL-1α in normal liver cells. Conversely, lower oestrogen levels and higher miR-22 expression levels in men downregulate ERα, which results in increased IL-1α expression and HCC development (76). Chen *et al* (78) treated HCC cells with IFN-γ to replicate the environment of hepatitis virus-associated HCC and reported that IFN-γ-induced Gal-9 expression in HCC cells was positively correlated with enhancer of zeste homolog 2 (EZH2) expression, which was significantly upregulated in both a concentration- and time-dependent manner. Data set analysis, quantitative PCR and western blotting showed that EZH2 inhibited miR-22 transcription and promoted Gal-9 expression in a DNA hypermethylation-independent manner. Consequently, EZH2's effect on GAL-9 is indirect, achieved through epigenetic repression of miR-22 (Fig. 1).

In conclusion, miR-22 may exhibit similar effects in hepatitis virus-associated HCC and other forms of HCC. However, it remains unclear why the level of miR-22 expression is much lower in patients with hepatitis virus-associated HCC and the differences in molecular mechanisms compared with non-hepatitis virus-induced HCC. Further research on whether and how the hepatitis virus affects the function of miR-22 in liver cancer is needed to explore these specific regulatory mechanisms.

Role of miR-22 in enhancing the therapeutic sensitivity of liver cancer cells. Due to the rise in resistance to the primary chemotherapy drug sorafenib, many patients with HCC who depend on sorafenib for their survival are unable to receive effective treatment and face a poor prognosis (79). This necessitates further research focussed on improving drug sensitivity in tumour cells and identifying new anti-tumour targets.

Cheng *et al* (80) reported that the levels of reactive oxygen species (ROS) and the redox state of sorafenib-resistant cells are inhibited, which creates a protective state for tumour cells, and SIRT1 can inhibit ROS production by regulating the expression of cellular antioxidant genes, thereby reducing tumour cell sensitivity to chemotherapeutic drugs. Lowering SIRT1 expression when treating HCC using chemotherapeutic drugs can help induce tumour cell death (81). Pant *et al* (54) reported the impact of SIRT1 inhibition on ROS release using 2',7'-dichlorofluorescein diacetate and showed that both butyrate and miR-22 triggered ROS release and miR-22 suppressed SIRT1 expression. However, when cells were co-incubated with butyrate and anti-miR-22, intracellular ROS production was significantly reduced, which suggests that miR-22 may enhance drug sensitivity by increasing tumour cell ROS levels, thereby promoting their death. miR-22-5p can enhance the radiosensitivity of HCC by increasing histone acetylation in the MIR22HG promoter region via radiolytic inhibition of HDAC2 activity (82). These findings suggest that miR-22 may increase the sensitivity of liver tumour cells to chemotherapy and radiotherapy, and to some extent, reverse their resistance to sorafenib and thus could potentially serve as a target for anti-tumour treatments.

Macrocytic anaemia is common in HCC and a previous animal study showed that lenvatinib, a first-line drug for liver cancer, may exacerbate anaemia, whereas miR-22 does not have this side effect (83). Instead, miR-22 increases white blood cell and platelet counts and could extend survival time (13).

Notably, the expression of miR-22 is lower in liver cancer induced by the hepatitis virus, and miR-22 can strongly inhibit the expression of the HBsAg and HBeAg. In addition, the regulation of miR-22 by HBx upregulating TRERNA1 expression promotes the resistance of HCC cells to sorafenib (14). Therefore, the exploration of the mechanism of action of miR-22 in viral hepatitis may be useful for the study of liver cancer treatment and drug resistance. Combining the diverse molecular mechanisms of miR-22 regulation in liver cancer and the regulation of the miR-22 pathway at the clinical level, in addition to actively exploring targets with similar biological effects may counteract the side effects and drug resistance observed with radiotherapy and chemotherapy treatments at present, and provide reference for the treatment and remission of liver cancer in the future.

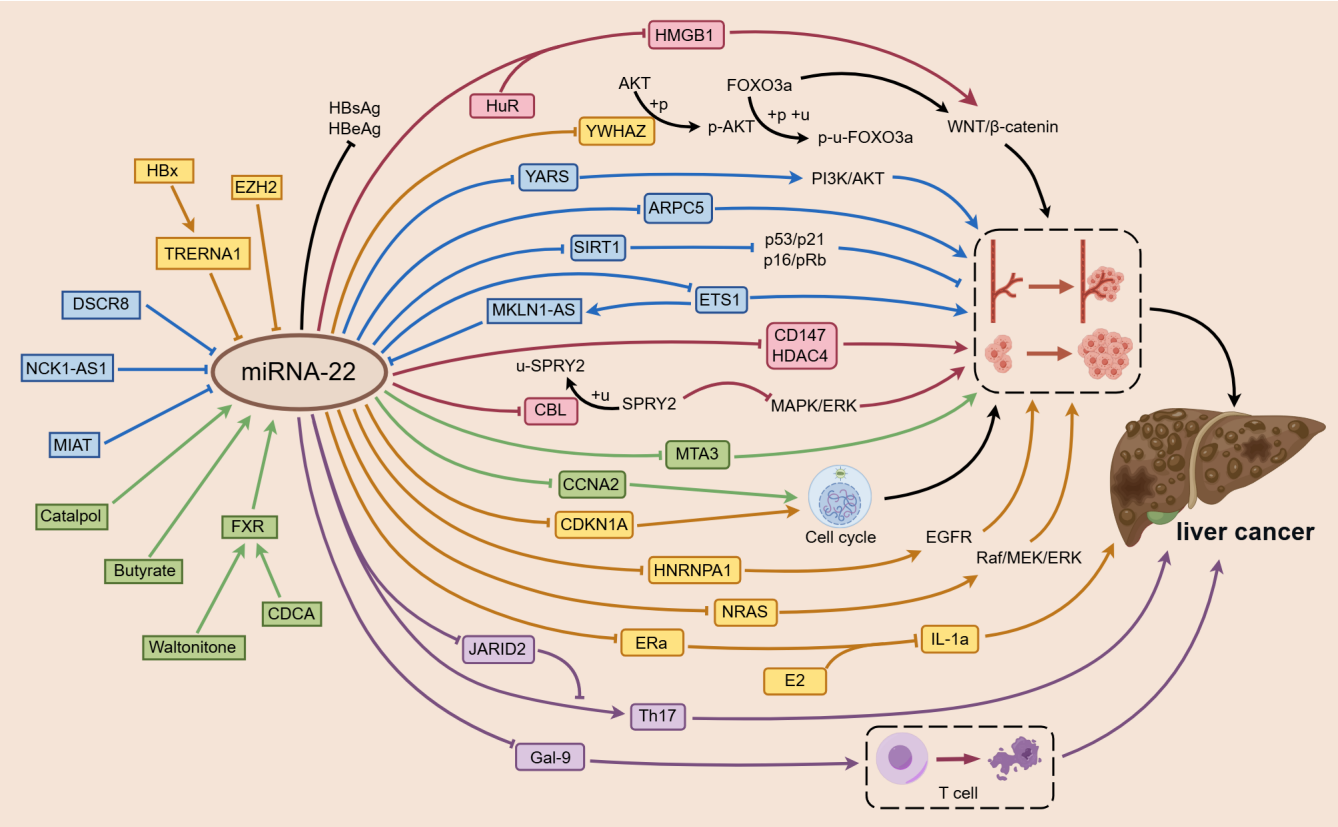

Figure 1. Mechanism underlying miR-22 regulation of the occurrence and development of HCC. miR-22 is involved in the regulation of the occurrence and development of HCC. miR-22 mainly inhibits the proliferation, migration and invasion of HCC by decreasing the expression levels of CD147 and HDAC4 and participating in the immunomodulatory process by regulating Gal-9 and Th17, which ultimately serves a role in cancer inhibition. lncRNAs, such as DSCR8, NCK1-AS1 and MKLN1-AS, exhibit sponging effects on miR-22, thereby weakening the inhibitory effect on downstream cancer-promoting factors ARPC5, YARS and ETS1. In hepatitis virus-associated liver cancer, the negative regulation of miR-22 by TRERNA1 and EZH2 also weakens the inhibitory effect on HNRNPA1 and NRAS, which promotes the occurrence and development of liver cancer. Exogenous substances and metabolites, such as catalpol, butyrate and FXR, induce the expression of miR-22, which enhances the regulation of miR-22 on downstream factors MTA3 and CCNA2 and inhibits the progression of HCC. An arrow-headed line indicates promotion, whereas a bar-headed line signifies inhibition. The rectangular box represents the upstream component of miR-22, and the circular corner box represents the downstream component regulated by miR-22. Yellow represents the mechanism of hepatitis virus-associated liver cancer, blue represents the mechanism related to lncRNA, green represents the mechanism related to the induction of miR-22 by endogenous and exogenous substances, purple represents the mechanism related to immunity and pink represents the mechanism of miR-22 regulating the proliferation and migration of liver cancer without the influence of other substances. The dashed line box on the right shows the proliferation and migration of liver cancer cells and the dashed line box below shows the lysis and death of Tim3+ T cells. EZH2, enhancer of zeste homolog 2; TRERNA1, translation regulatory lncRNA 1; HBx, HBV-encoded X; DSCR8, down syndrome critical region 8; NCK1-AS1, NCK1 antisense RNA 1; MIAT, myocardial infarction-associated transcript; FXR, farnesoid X receptor; CDCA, chenodeoxycholic acid; HuR, human antigen R; HMGB1, high mobility group box 1; YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; YARS, tyrosyl-tRNA synthetase; ARPC5, actin-related protein 2/3 complex subunit 5; SIRT1, sirtuin 1; ROS, reactive oxygen species; MKLN1-AS, muskelin 1 antisense RNA; ETS1, ETS proto-oncogene 1; CD147; HDAC4, histone deacetylase 4; CBL, casitas B-lineage lymphoma; MTA3, metastasis associated 1 family member 3; CCNA2, cyclin A2; CDKN1A, CDK inhibitor 1A; HNRNPA1, heterogeneous nuclear ribonucleoprotein A 1; NRAS proto-oncogene; ERα, estrogen receptor α; E2, estradiol; IL-1α, interleukin 1α; JARID2, jumonji AT rich interacting domain containing 2; Th17, T helper cell 17; Gal-9, galectin-9.

Early diagnosis and prognosis of miR-22 in liver cancer. Alpha fetoprotein (AFP) is an important marker in diagnosing and predicting the outcome of HCC as it presents at high levels in the serum of patients with liver cancer. However, AFP levels can also be elevated in pregnant women and patients with germ cell tumours (84). Therefore, relying solely on AFP for early HCC diagnosis is not highly specific or sensitive and is not recommended as a primary diagnostic method. Combining ultrasound with AFP can improve the rate of early liver cancer diagnosis (85), but some cases may still go undetected, which may lead to clinical diagnostic issues (86). A previous study reported that the expression of miR-22 was not only decreased in liver cancer tissues, but that the difference in its serum expression levels were also diagnostically significant (22). Zekri *et al* (87) demonstrated the detection methods combining miRNA markers such as miR-22, miR-885-5p, miR-221 and miR-122 in conjunction with AFP could be used to accurately diagnose HCC in patients with liver cirrhosis. This combination is particularly useful for the early diagnosis of HCC in patients with liver cirrhosis. The expression levels of miR-199-3p and miR-22 are significantly decreased in HCC and chronic hepatitis C infections. The aforementioned miRNAs, in addition to AFP, could potentially be used to assess the severity of chronic HCV infection and aid in diagnosing HCC resulting from HCV development.

Additionally, miR-122, miR-22, miR-99a and miR-125b expression levels are reported to be significantly higher in the serum of HBV patients compared with those in healthy

individuals. The expression levels are also significantly linked to HBV DNA levels (88). In chronic hepatitis B (CHB) infection, miR-22, when combined with miR-210 and ALT, can predict the virological and non-virological responses following IFN-α treatment, which is an antiviral agent used to treat CHB. This may be used to determine the efficacy of IFN-α treatment and reduce its adverse effects and complications (89). Furthermore, baseline serum exosomal miR-22-3p levels can forecast HBeAg seroconversion in patients with CHB undergoing Peg-IFN treatment (90).

Zhang *et al* (35) reported a positive correlation between miR-22 expression and the overall survival and disease-free survival in patients with HCC using bioinformatics methods, such as Kaplan-Meier analysis. Patients with HCC and normal or relatively high miR-22 expression had a better prognosis, which was consistent with the findings of another previous study (40). Chen *et al* (38) demonstrated that miR-22 was not only a predictor of prognosis but could also be used as an independent predictor of overall survival in patients with HCC. Therefore, assessing miR-22 expression levels in HCC tissues could be used to predict the prognosis of patients with HCC.

In summary, miR-22 could be used in the diagnosis and prognostic assessment of liver cancer, particularly for high-risk groups, such as patients with hepatitis virus infection and liver cirrhosis. As miR-22 can be used as an independent predictor of overall patient survival, a scientific detection and diagnosis system and a prediction model of liver cancer prognosis should be established to facilitate improved prognosis and survival for patients with liver cancer.

3. miR-22 in fatty liver diseases

The liver serves a crucial role in lipid metabolism and various metabolic disorders can arise if liver function is disrupted (91). When the liver is in a diseased state, it can lead to chronic metabolism-related conditions, such as fatty liver disease, which includes AFLD and NAFLD (92). miR-22 is involved in the development of both types of fatty liver disease (93,94). Alcoholic fatty liver diseases are closely linked to excessive alcohol consumption, which disrupts hepatic lipid metabolism pathways and leads to liver damage. miR-22 inhibitors can be used to help improve alcohol-induced steatosis (95). NAFLD, is associated with certain metabolic disorders, including obesity and diabetes mellitus, and is characterized by the accumulation of fat and steatosis in the liver (96). Additionally, excessive glycogen accumulation has been identified as a key factor in the development of liver malignant transformation, as reported by Liu *et al* (97). The progression from NAFLD to HCC is primarily caused by the excessive accumulation of fat in the liver. This leads to abnormal signalling pathways, which results in liver cell injury and chronic inflammation. This process significantly increases the risk of developing liver fibrosis and HCC (98). In the case of NAFLD, inhibitors of miR-22 improve hepatic steatosis and reduce fat build-up in the liver by regulating factors involved in fatty acid metabolism (94). Therefore, miR-22 inhibitors show promise as potential future therapeutic agents for managing hepatic steatosis in fatty liver disease.

AFLD. Excessive alcohol consumption can lead to a condition known as alcoholic liver disease (ALD), which causes injury to the liver. In alcohol metabolism, by-products can have toxic effects on the liver, which can ultimately result in ALD (99). This initially presents as alcoholic steatosis, which can progress to steatohepatitis, hepatic fibrosis, cirrhosis and has the potential to develop into HCC. ALD is closely linked to hepatic steatosis, with alcohol affecting hepatic lipid metabolism by altering how the liver takes up lipids, synthesizes lipids, oxidizes fatty acids, exports lipids, forms lipid droplets and undergoes catabolism (100). A previous study reported a positive relationship between β-catenin and miR-22 expression levels. Inhibition of β-catenin activity decreases miR-22 expression (93). Chronic alcohol intake activates β-catenin and increases the expression levels of miR-22-3p, which in turn, inhibits tet methylcytosine dioxygenase 2 (TET2) and promotes HCC stemness and metastasis. In HCC, TET2 expression is reduced and alcohol exposure further increases miR-22-3p expression levels, which leads to a decrease in TET2 expression. This promotes tumour growth and metastasis in HCC cells. Therefore, the β-catenin/miR-22-3p/TET2 axis serves a role in alcohol-induced HCC malignant progression.

The fibroblast growth factor 21 (FGF21) signalling pathway is responsible for maintaining liver metabolic balance (101) and utilizes FGF21 receptor (FGFR1) as its receptor. FGFR1 deficiency diminishes FGF21 signalling in adipocytes, therefore FGF21 and FGFR1 are the primary targets and regulators of certain metabolic diseases. Key transcription factors for liver FGF21 are hepatic PPAR-activated receptor-c coactivator-1α (PGC1α) and peroxisome proliferator-activated receptor α (PPARα). In cases of fatty liver, the expression levels of miR-22, FGF21, FGFR1 and PGC1α are inversely correlated. Hu *et al* (95) reported that the expression levels of FGFR1 and FGF21 decreased in Huh7 cells after treatment with miR-22, which indicates the presence of a relationship between miR-22 and FGF21 signal transduction. The study also reported that miR-22 could directly target and reduce FGFR1. Additionally, miR-22 reduces the expression levels of FGF21 by reducing the regulation of transcription factors PPARα and PGC1α, thereby limiting the activation of ERK 1/2 and promoting fat accumulation. Inhibiting miR-22 increases FGF21 and FGFR1 levels in the liver, which strengthens the FGF21 signal transduction pathway in the liver leading to the activation of AMPK and ERK1/2, thus promoting lipid metabolism in alcoholic fatty liver and improving alcohol-induced steatosis. Therefore, miR-22 inhibitors can be used to increase FGF21 and FGFR1 levels and treat liver steatosis. In addition, the miR-22 inhibitor was as effective as obeticholic acid in treating steatosis and reducing the accumulation of liver fat. Combined treatment with the two drugs significantly improves insulin sensitivity, releases glucagon-like peptide 1 and reduces liver triglycerides in obese mice.

Summarily, the efficacy of miR-22 in improving alcohol-induced steatosis has been previously reported. Studies have shown that mice injected with anti-miR-873-5p have relatively high SIRT1 activity in their liver, which can delay the progress of alcoholic liver disease by enhancing the activity of SIRT1 deacetylase (102). Iwagami *et al* (103) reported that SIRT1 may be a key contributing factor for the actions of miR-34a to reverse alcoholic fatty liver. In

view of the complicated mechanism of regulation of SIRT1 by miR-22 in liver cancer, investigating whether miR-22 can improve AFLD by regulating SIRT1 is particularly important. Although studies on miR-22 and alcoholic fatty liver are currently scarce, the close relationship between miR-22 and alcoholic fatty liver cannot be disregarded. In future, research in this field will enable the understanding of the specific regulatory mechanism of miR-22 in alcoholic fatty liver.

NAFLD. NAFLD is a clinicopathological syndrome characterized by the accumulation of fat in the liver (104). Patients with NAFLD show liver manifestations of metabolic syndrome, including fatty degeneration of the liver observed using imaging techniques and histology (105). Several experimental studies have demonstrated the role of miR-22 in NAFLD (23,94,106).

Yang *et al* (94) investigated the genes involved in regulating fat metabolism by miR-22. The authors induced obesity in a mouse model using a high-fat diet (HFD) and treated a normal human liver cell line with free fatty acids to stimulate fat accumulation in liver cells. The study reported increased expression levels of miR-22 in the obese mouse model and human liver cells exposed to fatty acids and overexpressing miR-22 resulted in fat accumulation in the liver cells. PPARα and Sirt1 are involved in fatty acid metabolism and miR-22 interacts with Sirt1 to participate in liver fat metabolism (25,94). In the fatty acid-induced human hepatocyte line, L02, miR-22 expression levels were increased and Sirt1 expression levels were decreased compared with the non-induced L02 cells. Sequence analysis showed that miR-22 can directly interact with the 3'-UTR of Sirt1 to regulate lipid metabolism and their expression levels were negatively correlated. miR-22 analogues significantly reduced the expression levels of PPARα and FOXO1, while miR-22 inhibitors notably increased their expression levels. This suggests that miR-22 serves a role in regulating a series of downstream genes related to fatty acid metabolism. miR-22 inhibitors enhance the expression of genes related to fatty acid metabolism, which reduces lipid accumulation in the liver. Therefore, in the HFD-induced mouse model, the upregulation of miR-22 expression is involved in regulating lipid metabolism, energy balance and obesity. Decreasing expression of the miR-22 gene can increase energy consumption and disrupt lipid biosynthesis.

Thibonnier and Esau (107) reported that when the miR-22 antagonist APT-110 was introduced into human subcutaneous preadipocytes, important factors for metabolism, such as mitochondrial activity, uncoupling protein 1 expression and energy expenditure, were increased. Additionally, after subcutaneous injection of miR-22-3p antagonist APT-110 into the groin of mice fed a HFD, a notable increase in metabolic and lipolysis rates, accompanied by significant decreases in blood sugar, plasma insulin and leptin levels were reported. Administration of APT-110 resulted in a significant reduction in the overall weight gain and average liver fat content in HFD mice compared with the control group (saline injection). Inhibiting miR-22-3p led to a reduction in genes related to the fatty acid biosynthesis pathway in the liver, while generally increasing genes associated with fatty acid metabolism in inguinal fat, thereby reducing liver fat accumulation (108). These findings suggest miR-22 inhibitors may be a promising new future approach for controlling obesity and fatty degeneration of hepatocytes.

Panella *et al* (23) developed a mouse model with a miR-22 transgene controlled by Cre recombinase and reported that mice carrying the miR-22 transgene gained weight quickly and showed high levels of miR-22 in the liver tissue. The increased miR-22 expression levels in the liver induced fatty degeneration. Additionally, the authors investigated the role of miR-22 in metabolism by targeting mice with liver tissue-specific miR-22 knock-out. Compared with wild-type mice, the miR-22 knock-out mice gained significantly less weight after 8 weeks on a HFD and displayed reduced liver steatosis. Immunohistochemistry results showed increased staining of the uncoupling protein 1 in miR-22 knock-out mice and these mice also exhibited white fat browning. These findings indicate that knocking out miR-22 can decrease liver cell fatty degeneration and obesity in mice under HFD conditions.

Gjorgjieva *et al* (109) investigated the role of miR-22 in liver lesions in mice fed with an HFD. The study used a model of mice with a knocked-out miR-22 gene (miR-22KO) and fed them a HFD for 12 weeks, which led to an increase in fat mass, hepatomegaly and hepatic steatosis. These findings suggest the importance of miR-22 in liver diseases. To further explore the link between obese mice with miR-22 deficiency and the development of liver cancer, Gjorgjieva *et al* (39) also published a study using miR-22KO mice. Feeding the mice a HFD resulted in the promotion of characteristic features of nonalcoholic steatohepatitis (NASH), which included liver changes resembling balloon-like structures. Diethylnitrosamine was administered to miR-22KO and wild-type mice to induce liver cancer and the animals were divided into two groups, where one group received a standard diet and the other received an HFD. These results indicated that the miR-22KO mice developed tumours earlier and the HFD group had a high number of tumours with low differentiation characteristics compared with the control group. This suggests that NAFLD can worsen tumour development and differentiation in mice with miR-22 deficiency. A comparison of the research results from the aforementioned study and those reported by Panella *et al* (23) showed the complex metabolic regulatory role of miR-22 in the body, which indicates a need for further research.

NAFLD is a systemic metabolic disease mainly caused by obesity and type 2 diabetes mellitus (T2DM). T2DM can accelerate the progression of NAFLD in liver disease. Worldwide, ~55.48% of patients with T2DM have NAFLD and among those patients undergoing liver biopsy, 37.33% have NASH and 17.02% have advanced fibrosis (110), which highlights the close relationship between diabetes and NAFLD. A previous study reported that miRNAs serve a crucial role in insulin signalling transduction, glucose metabolism regulation, HDL and LDL homeostasis regulation and liver lipid metabolism (111). miR-22 is highly expressed in the liver and regulates liver metabolism in disease states such as diabetes. In mice with insulin resistance and T2DM, miR-22 expression levels are significantly increased and liver glucose metabolism is regulated by targeting the transcription factor 7 (TCF7) in the Wnt pathway. Silencing miR-22 improves circulating glucose and insulin levels and reduces fasting blood glucose levels in mice (112). Additionally, 3,5-diiodine-L-thyronine (T2) serves a role in increasing the resting metabolic rate as well as lipid and glucose metabolism (113,114). miR-22 serves a prominent role in T2 metabolism and is involved in the homeostasis of

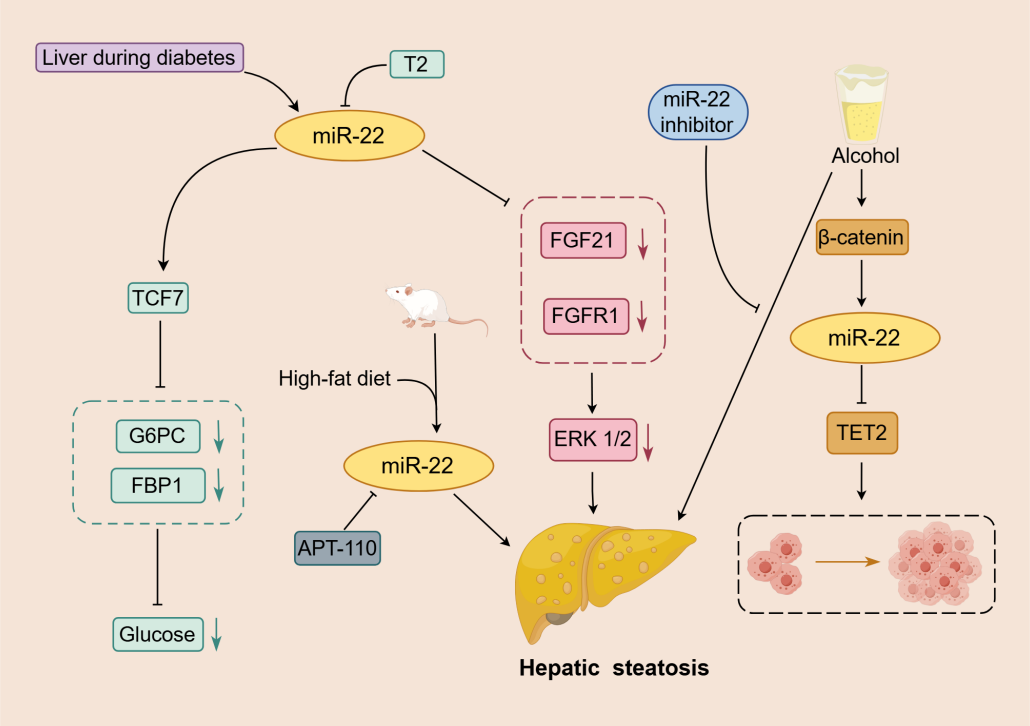

Figure 2. Mechanism of action of miR-22 in fatty liver disease. miR-22 is involved in regulating the progression of fatty liver disease. Increased expression of miR-22 in liver tissue is observed with long-term alcohol intake, high-fat diet and diabetes. miR-22 targets and inhibits FGFR1, FGF21 and TET2, which contributes to hepatic steatosis and the progression of HCC. Moreover, T2 upregulates TCF7 by downregulating miR-22, which subsequently suppresses the expression of FBP1 and G6PC, and regulates glucose homeostasis in the liver. An arrow-headed line indicates promotion, whereas a bar-headed line signifies inhibition. The yellow box indicates miR-22. Other boxes of the same color represent important factors in the same pathway. The green and red dotted boxes represent important factors in their respective pathways. The black dotted box indicates HCC cell proliferation. FBP1, fructose 1-6 bisphosphatase; G6PC, glucose 6-phosphatase; miR, microRNA; TCF7, transcription factor 7; T2, 3,5-diiodine-L-thyronine; TET2, tet methylcytosine dioxygenase 2; FGF21, fibroblast growth factor 21; FGFR1, FGF21 receptor.

glucose metabolism by T2. T2 downregulates miR-22 to upregulate its target TCF7, impairs glucose production by inhibiting the expression of glucose-producing enzyme and regulates glucose homeostasis (115). The liver can also regulate glucose homeostasis through various pathways that control glucose metabolism. Mebhydrolin, a selective nuclear receptor FXR antagonist, reduces miR-22-3p expression levels by antagonising FXR. This inhibits hepatic gluconeogenesis through the FXR/miR-22-3p/PI3K/AKT/FOXO1 pathway and promotes glycogen synthesis via the FXR/miR-22-3p/PI3K/AKT/GSK-3β pathway to improve blood glucose homeostasis in T2DM mice (116). Therefore, miR-22 may act as an indicator to predict physiological and pathological changes in the liver during T2DM.

Summarily, the expression levels of miR-22 increase after high fat induction, and fatty degeneration of liver can be induced by regulating fatty acid metabolism. The role of the miR-22 inhibitor in improving energy consumption and reducing liver fat accumulation has been reported. In addition, in view of the close relationship between glucose metabolism and NAFLD, as well as the complex regulatory role and differential correlation of miR-22 in NAFLD, further exploration of the regulatory mechanism of miR-22 is necessary. Future research is expected to reveal additional new targets of miR-22 regulating hepatic steatosis and provide potential new strategies for the future treatment of fatty liver diseases. In addition, the global prevalence of NAFLD is likely to increase in the future and the role of miR-22 in NAFLD is expected to become more prominent (10). Improvements in miR-22-related detection methods and the development of miR-22-related preparations will contribute to the early detection and treatment of fatty liver disease in the future (Fig. 2).

Functional differences of miR-22 in AFLD and NAFLD. miR-22 serves a central role in the pathogenesis of both AFLD and NAFLD. Despite the common features between the two diseases, there are obvious differences in the specific functional manifestations and underlying molecular regulatory mechanisms. miR-22 is upregulated in the fatty liver caused by two different triggers, excessive alcohol consumption is the key to triggering AFLD, while obesity, hyperlipidemia and type 2 diabetes are important factors in NAFLD, and the overexpression of miR-22 aggravates the degree of hepatic steatosis (94,95). In AFLD, the level of miR-22 is positively correlated with alcohol consumption and inhibits the FGF21/FGFR1 signaling pathway to promote the development of fatty liver by silencing PPARα (95). In NAFLD, miR-22 is associated with a HFD and directly regulates Sirt1, PPARα and FOXO1 expression to promote the formation of fatty liver (94). Therefore, PPARα serves a key role in the regulation of miR-22. Additionally, miR-22 also participates in the regulation of hepatic gluconeogenesis by regulating TCF7 in the Wnt pathway, a process that may be inhibited by T2 (112,115), and maintains blood glucose homeostasis through interacting with complex networks such as FXR/miR-22-3p/PI3K/AKT (116),

which affects the development of NAFLD (Table I). Further, miR-22 inhibitors have significant effects on improving both alcohol- and non-alcohol-induced steatosis. In AFLD, miR-22 inhibitors activate AMPK by upregulating the expression of FGF21 and FGFR1 to improve alcohol-induced steatosis (95), whereas in NAFLD, it also reduces steatosis and weight gain by increasing total energy expenditure and improving insulin sensitivity (107). This similar, yet different, characteristic deepens the understanding of the complex role of miR-22 in the process of hepatic steatosis and also provides important perspectives which are useful for exploring targeted therapeutic strategies.

In conclusion, miR-22 serves a significant role in the fatty liver. In the future, it is necessary to further explore its interaction mechanism with target genes and its impact on pathophysiology, accelerate the development of miR-22 inhibitors and provide new strategies for treatment. Additionally, in view of the differences in mechanisms between AFLD and NAFLD, treatment should be personalized, multi-target combination therapy should be consider and combined with lifestyle interventions. Research into the mechanisms of action of miR-22 provide a new perspective for the treatment of fatty liver and further in-depth research and drug development is required to improve the efficacy of patient treatment.

4. Role of miR-22 in liver fibrosis

Liver fibrosis can be caused by different factors, such as viral hepatitis, alcoholic steatohepatitis, non-alcoholic steatohepatitis and DILI (117). These factors lead to the induction of the liver repair response, which results in increased liver extracellular matrix (ECM) and the formation of fibrous scars (118). Currently, effective drug treatments for liver fibrosis are lacking. It is an important step in the transition from chronic liver disease to cirrhosis and is characterized by hepatic stellate cell (HSC) activation and excessive ECM deposition (119). If not treated promptly and effectively, liver fibrosis can progress to cirrhosis, liver failure and potentially liver cancer. HSC activation significantly influences the occurrence and development of liver fibrosis, with various miRNAs having the ability to regulate liver fibrosis signalling pathways and HSC activation (120). Each miRNA exerts distinct regulatory effects. For example, miR-188-5p enhances HSC activation and proliferation through the PTEN/PI3K/AKT pathway, thereby promoting liver fibrosis (121). In addition, miR-301a-3p promotes HSC activation and liver fibrosis through the PTEN/PDGFR-β pathway (122). However, miR-22 can inhibit HSC activation and the expression of related fibrotic mediators in various ways, thereby mitigating the progression of liver fibrosis. Collectively, miRNA has great research potential and value in the treatment of liver fibrosis.

miR-22 is closely associated with HSC activation and liver fibrosis. Huang *et al* (27) reported that the lncRNA nuclear paraspeckle assembly transcript 1 (Neat1) functions as a ceRNA to accelerate the progression of liver fibrosis in mice by targeting miR-148a-3p and miR-22-3p, thereby upregulating cytohesin 3. Conversely, downregulating Neat1 yielded contrasting results. The inhibitors for miR-22-3p and miR-148a-3p stimulate the activation of HSCs and the expression of collagen fibres, which can lead to liver fibrosis.

AKT3, is a serine/threonine protein kinase, whose expression level is regulated by the miRNA (123-125). AKT3 is a common target gene of miR-22-3p and miR-29a-3p, promoting the proliferation, migration, colony formation ability and the expression of fibrosis markers collagen type I α 1 chain and α-smooth muscle actin in LX-2 cells (24,123). Under the influence of miR-22-3p and miR-29a-3p inhibitors, the expression of AKT3 increases, thereby promoting the proliferation and activation of LX-2 cells. In conclusion, the overexpression of miR-22-3p and miR-29a-3p synergistically inhibits the proliferation and activation of LX-2 cells and alleviates the progression of liver fibrosis (24).

Silymarin (SIL)-loaded chitosan nanoparticles combine chitosan nanoparticles with the hepatoprotective compound SIL to enhance its therapeutic effect in liver diseases and improve its anti-fibrotic efficacy in CCl rats. The mechanism of action involves promoting the expression of protective factors miR-22, miR-29c and miR-219a in the liver, which in turn inhibit the expression of fibrosis mediators TGFβR1, TGFβR2 and collagen type III α1 chain, thus slowing down the progression of liver fibrosis (126). Additionally, Abdullah *et al* (127) demonstrated that SIL-gold nanoparticles serve a similar role in the process of liver fibrosis. In summary, miR-22 acts as a protective factor in the liver, inhibiting the expression of fibrotic mediators and serving an anti-fibrotic role in liver fibrosis.

NAFLD speeds up the process of liver fibrosis caused by carbon tetrachloride. In turn, liver fibrosis accelerates the progression of liver cancer (128). NAFLD and liver fibrosis have a significant impact on the occurrence and development of liver cirrhosis and HCC. A study by Ji *et al* (129) reported that the expression levels of miR-22 were negatively correlated with bone morphogenetic protein 7 (BMP7) in the liver biopsies of 12 patients with liver cirrhosis and this conclusion was verified in HepG2 cells. Bioinformatics analysis of the target sequence of BMP7 shows that miR-22 can target the 3'UTR of BMP7 mRNA and inhibit the expression of BMP7, which leads to the occurrence of liver cirrhosis (129). miR-22 was delivered to the liver through the common bile duct, thus effectively reducing the potential interference of other tissues and organs on the experimental results.

Although the role of miR-22 in the process of liver fibrosis has been reported to a certain extent, numerous potential mechanisms remain unclear, particularly in the study of drug transformation. In addition, significant individual differences exist in the clinical manifestations and severity of illness among patients during the progression of liver fibrosis to cirrhosis and whether the expression levels of miR-22 also differ has not yet been determined. Therefore, it is crucial to identify and implement therapeutic measures during the early stages of the disease. It is also necessary to explore the underlying molecular mechanisms, understand the formation and development of the disease and identify appropriate therapeutic targets and treatment measures. Simultaneously, a reasonable model for managing chronic liver disease should be developed to effectively control its malignant progression (Fig. 3).

5. miR-22 and DILI

DILI is a rare but serious drug-induced adverse reaction, which can lead to the early termination or withdrawal of drug development

Table I. Key roles of miR-22 in different types of liver lesions.

A, liver cancer

Experimental model	*In vivo* or *in vitro*	Target	Function	(Refs.)
Human liver tissues and Hep3B and SMMC7721 cells	*In vivo* and *in vitro*	HDAC4	miR-22 acts on CD147, HDAC4, HMGB1, CBL and other targets to directly or indirectly regulate the proliferation, migration, invasion and apoptosis of HCC cells	(35)
Human liver tissues and SK-Hep-1 and SMMC-7721 cells	*In vivo* and *in vitro*	HMGB1		(43)
Murine xenograft model and MHCC-97H and SMMC-7721	*In vivo* and *in vitro*	CD147		(44)
HepG2 and Huh7 cells	*In vivo*	CBL		(17)
Model of subcutaneous tumors in nude mice (male BALB/c nude mice aged 4-5 weeks) and Hep3B and Huh7 cells	*In vivo* and *in vitro*	ARPC5	miR-22 is negatively regulated by lncRNAs, and its inhibitory effect on downstream targets is weakened, which results in the promotion of growth and migration of liver cancer	(37)
Xenograft tumor model (BALB/c male nude mice), human liver tissues and Huh7, Hep3B, MHCC97-H and LM3 cells	*In vivo* and *in vitro*	ETS1		(49)
Human liver tissues and Hep-3B, Huh7, HepG2, SK-Hep1 and L02 cells	*In vivo* and *in vitro*	YARS		(52)
Subcutaneous tumor model (female BALB/c mice aged 6-8 weeks) and HepG2, SMMC7721, Huh7 and SK-HEP-1 cells	*In vivo* and *in vitro*	SIRT1		(47)
In vivo xenograft model of human HCC nude mice (male, aged 5 weeks) and Huh7 and HCCLM3 cells	*In vivo* and *in vitro*	MTA3	After miR-22 is induced by Catalpol and FXR, miR-22 restricts the proliferation, migration and invasion of HCC cells by affecting the normal cell cycle	(41)
Human liver tissues, mouse model of liver cancer and Huh7 cells	*In vivo* and *in vitro*	CCNA2		(36)
Human liver tissues and HepG2.2.15 cells	*In vivo* and *in vitro*	CDKN1A	In the hepatitis virus-related liver cancer model, miR-22 exerts an inhibitory effect on downstream targets, which hinders the occurrence and development of hepatitis virus-related liver cancer	(71)
Human liver tissues and MHCC97H, Hep3B, HepG2.2.15, Huh7 and L02 cells	*In vivo* and *in vitro*	HNRNPA1		(70)
Human liver tissues and L02, MHCC97L and HCCLM9 cells	*In vivo* and *in vitro*	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein zeta		(38)

Table I. Continued.

A, liver cancer

Experimental model	*In vivo* or *in vitro*	Target	Function	(Refs.)
Human liver tissues and HepG2, HepG2.215, Hep3B and L02 cells	*In vivo* and *in vitro*	NRAS		(14)
Human liver tissues, immunoreconstituted mouse model (female C57BL/6 mice aged 8-10 weeks) and $CD4^+$ T cells	*In vivo* and *in vitro*	JARID2 and T helper17cells	miR-22 hinders the occurrence and progression of tumors by exerting immunomodulatory effects, including inhibiting tumor growth and immune escape and regulating the apoptosis of tumor cells.	(43)
Human liver tissues and Lo2, HepG2 and SMMC7721 cells	*In vivo* and *in vitro*	Galectin-9		(66)

B, alcoholic fatty liver disease

Experimental model	*In vivo* or *in vitro*	Target	Function	(Refs.)
C57BL/6 wild-type male and female mice and Huh7 cells	*In vivo* and *in vitro*	FGF21 receptor	miR-22 reduces FGF21 expression by reducing the regulation of FGF21 by the transcriptional factors PPARα and hepatic PPAR-activated receptor-c coactivator-1α, which limits AMPK and ERK1/2 activation, leading to fat accumulation	(95)

C, nonalcoholic fatty liver disease

Experimental model	*In vivo* or *in vitro*	Target	Function	(Refs.)
Male C57BL/6 mice and L02 cells	*In vivo* and *in vitro*	Sirt1, PPARα and FOXO1	miR-22 regulates the expression of Sirt1, PPARα and FOXO1 to regulate lipid metabolism	(94)
Male diabetic (C57BLKs-db/db) and non-diabetic (C57BLKs-db/+) mice and HepG2 cells	*In vivo* and *in vitro*	TCF7	miR-22 targets the transcription factor TCF7 in the Wnt pathway to regulate hepatic glucose gluconeogenesis	(112)
C57BL/6 mice, male db/db mice and HEK293T cells	*In vivo* and *in vitro*	FXR	miR-22 inhibits hepatic gluconeogenesis and promotes glycogen synthesis to maintain blood glucose homeostasis through the FXR/miR-22-3p/PI3K/AKT/FOXO1 and FXR/miR-22-3p/PI3K/AKT/GSK-3β pathways	(116)
Male Wistar rats	*In vivo*	TCF7	3,5-diiodine-L-thyronine significantly decreases the expression of miR-22, which results in a decrease in the inhibitory effect of miR-22 on TCF7, thus impairing gluconeogenesis	(115)

Table I. Continued.

D, liver fibrosis

Experimental model	*In vivo* or *in vitro*	Target	Function	(Refs.)
Male C57BL/6J mice and HSCs	*In vivo* and *in vitro*	Cyth3	Nuclear paraspeckle assembly transcript 1 is highly expressed in mouse liver tissues and acts as a competing endogenous RNA targeting miR-22, upregulating Cyth3 which is involved in HSC activation and liver fibrosis	(27)
Sprague-Dawley male rats, NFs and LX-2 cells	*In vivo* and *in vitro*	AKT3	miR-22 significantly inhibits the proliferation and activation of LX-2 cells and alleviates liver fibrosis	(24)

E, drug-induced liver injury

Experimental model	*In vivo* or *in vitro*	Target	Function	(Refs.)
C57BL/6 mice	*In vivo*	NA	Mouse autoimmune hepatitis is induced using concanavalin A and miR-22 expression is downregulated	(136)
HepG2 cells	*In vitro*	NA	The expression of miR-22 in HepG2 cells is up-regulated under the influence of the steatogenic drug cyclosporine A	(137)

NA, not applicable; miR, microRNA; HDAC4, histone deacetylase 4; HMGB1, high mobility group box 1; CBL, casitas B-lineage lymphoma; ARPC5, actin-related protein 2/3 complex subunit 5; ETS1, ETS proto-oncogene 1; YARS, tyrosyl-tRNA synthetase; lncRNA, long non-coding RNA; SIRT1, sirtuin 1; MTA3, metastasis associated 1 family member 3; CCNA2, cyclin A2; CDKN1A, CDK inhibitor 1A; HNRNPA1, heterogeneous nuclear ribonucleoprotein A 1; NRAS, NRAS proto-oncogene; FGF21, fibroblast growth factor 21; PPARα, peroxisome proliferator-activated receptor α; TCF7, transcription factor 7; FXR, farnesoid X receptor; Cyth3, cytohesin 3; HSC, hepatic stellate cells; HCC, hepatocellular carcinoma; NFs, normal fibroblasts; JARID2, jumonji AT rich interacting domain containing 2.

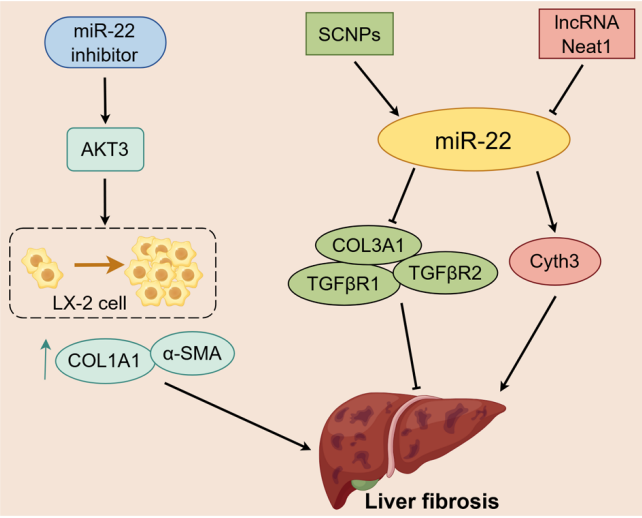

Figure 3. Mechanism of miR-22 in liver fibrosis. miR-22 is involved in regulating the progression of liver fibrosis. The expression level of miR-22 is controlled by the hepatoprotective complex SCNPs and lncRNA Neat1. miR-22 targets and regulates the expression of fibrosis mediators TGFβR1, TGFβR2, COL3A1 and Cyth3 to mitigate the advancement of liver fibrosis. Inhibition of miR-22 can enhance the expression of AKT3 in the liver, stimulate the proliferation of LX-2 cells and the expression of fibrosis markers COL1A1 and α-SMA and accelerate liver fibrosis. An arrow-headed line indicates promotion, whereas a bar-headed line signifies inhibition. The yellow box indicates miR-22. Other boxes of the same color represent important factors in the same pathway. The black dotted box indicates the process of LX-2 cell proliferation. miR, microRNA; COL1A1, collagen type I a1 chain; a-SMA, a-smooth muscle actin; SCNPs, SIL-loaded chitosan nanoparticles; lncRNA, long non-coding RNA; COL3A1, collagen type III a1 chain; TGF-bR, TGF-b receptor; Cyth3, cytohesin 3; neat1, nuclear paraspeckle assembly transcript 1.

studies (130). DILI is responsible for a growing number of liver injuries in previous years (131). The pathology of DILI-induced liver injuries are varied and complex and cases of DILI present as diverse histological types on liver biopsies (132). Liver biopsies of 249 patients with suspected DILI showed predominantly acute and chronic hepatitis (133). miRNAs are closely associated with the regulation of biological behaviours in various diseases. The role of miR-122 in DILI has been previously reported and it shares similarities with that of miR-22 in liver-related diseases. Both miRNAs inhibit HCC progression, facilitate NAFLD progression and detect hepatic fibrosis severity (134,135). Previous studies have demonstrated the main functions of miR-22 in the liver under drug induction (136,137).

Pharmacological autoimmune hepatitis is a type of liver injury induced by a drug or its metabolite, which triggers the immune response against foreign substances. The role of miRNAs in autoimmune hepatitis has been previously reported. Liu *et al* (136) used concanavalin A to induce autoimmune hepatitis in mice and the abnormal expression of various miRNAs in autoimmune hepatitis was analysed using gene microarray, enrichment analysis of Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) The expression level of miR-22 was downregulated, which provided new insights into the role of miR-22 in autoimmune hepatitis. This may potentially serve as a predictor of autoimmune hepatitis pathogenesis and a future therapeutic target for this disease.

Drug-induced hepatic steatosis is also a form of DILI; however, it is usually a reversible form of chronic disease (138). Obesity and NAFLD may increase the risk of hepatotoxicity of certain drugs and potentially exacerbate DILI (139). López-Riera *et al* (137) treated HepG2 cells with steatosis-mimicking drugs, such as doxycycline and cyclosporine A, and identified a group of miRNAs, including miR-22, that were induced in human HepG2 cells. The expression levels of miR-22 increased inside the cells and was released outside the cells and elevated levels of miR-22 were detected in the culture medium. The production of related miRNAs, including miR-22, was also induced when cells were exposed to prescription drugs, such as irbesartan and fenofibrate, which are used for NAFLD treatment (137). In addition, these miRNA biomarkers were detected in the sera of patients with NAFLD and their expression was significantly increased. Therefore, miR-22 may potentially be promising serum miRNA biomarker for drug-induced steatosis for drug development and screening.

Although the specific molecular mechanism of miR-22 in DILI remains unclear, bioinformatics analysis of related studies indicates its abnormal expression. Further research is needed to elucidate the molecular biological mechanism of miR-22 in DILI. In view of its abnormal expression in drug-induced liver injury, the feasibility of miR-22 as a potential miRNA marker in serum needs to be fully verified through in-depth research and clinical studies. Therefore, miR-22 could act as an important biomarker for the early diagnosis and prognosis evaluation of drug-induced liver injury in clinical practice in the future.

6. Summary and outlook

As a member of the miRNA family, miR-22 can bind to the 3'-UTR of target genes and regulate the expression of related genes, serving certain biological functions in various types of tumours. miR-22 predominantly acts as a tumour suppressor in numerous types of cancer, but under specific circumstances, it can act as a tumour promoter. The diverse molecular mechanisms of miR-22 on the regulation of liver cancer from numerous perspectives were comprehensively reviewed. Additionally, the role of miR-22 in the regulation on cell proliferation and cell cycle, immune regulation, sensitivity to treatment of liver cancer and evaluation of prognosis were discussed. miR-22 serves an oncogenic role by inhibiting the activities of liver cancer cells, such as proliferation, migration and invasion, while promoting apoptosis and participating in immunomodulation. miR-22 can be sponged and inhibited by various lncRNAs and induced by certain exogenous substances. Combined with the latest research of miR-22 in fatty liver disease, liver fibrosis and drug-induced liver injury, significant differences in the expression of miR-22 in different stages of liver diseases were reported, as well as the complex and subtle regulatory mechanisms underlying these differences. Therefore, the regulatory role of miR-22 in the pathological and physiological changes of the liver could be particularly important and may provide a novel research target for the diagnosis, treatment and prognosis prediction of liver diseases in the future. The present manuscript highlighted the necessity for further exploration of the detailed mechanisms of action of miR-22 in liver diseases. However, comprehensive

studies remain warranted to fully understand the complex regulatory functions of miR-22 in different types of tumours.

While the regulatory role of miR-22 in liver cancer has been extensively studied, questions still remain regarding its involvement in hepatitis virus-induced liver cancer. Furthermore, the mechanisms underlying DILI and the specific regulatory relationship between diabetes and fatty liver have yet to be fully understood, in addition to the multifaceted regulatory functions of miR-22 in various parts of the body. Further study into these areas will facilitate the development of agonists, inhibitors or drug combination therapies to utilize the complex regulatory functions of miR-22 in liver lesions and of miR-22 as a screening indicator or a prognostic model for liver lesions (Fig. S1).

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Authors' contributions

MW and XW consulted relevant research, searched the literature and participated in the writing of manuscripts and charts. YW, YG, JY and XX participated in the editing of the article. XY provided constructive guidance and revised the article. Data authentication is not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Asrani SK, Devarbhavi H, Eaton J and Kamath PS: Burden of liver diseases in the world. J Hepatol 70: 151-171, 2019.
2. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E and Kamath PS: Global burden of liver disease: 2023 Update. J Hepatol 79: 516-537, 2023.
3. Boldo E, Santafe A, Mayol A, Lozoya R, Coret A, Escribano D, Fortea-Sanchis C, Muñoz A, Pastor JC, Perez de Lucia G and Bosch N: Rare site hepatocellular carcinoma metastasis. J Hepatocell Carcinoma 7: 39-44, 2020.
4. Singal AG, Kudo M and Bruix J: Breakthroughs in hepatocellular carcinoma therapies. Clin Gastroenterol Hepatol 21: 2135-2149, 2023.
5. Gao R, Kalathur RKR, Coto-Llerena M, Ercan C, Buechel D, Shuang S, Piscuoglio S, Dill MT, Camargo FD, Christofori G and Tang F: YAP/TAZ and ATF4 drive resistance to sorafenib in hepatocellular carcinoma by preventing ferroptosis. EMBO Mol Med 13: e14351, 2021.
6. Bodzin AS and Busuttil RW: Hepatocellular carcinoma: Advances in diagnosis, management, and long term outcome. World J Hepatol 7: 1157-1167, 2015.
7. Zhang Y, Shi ZL, Yang X and Yin ZF: Targeting of circulating hepatocellular carcinoma cells to prevent postoperative recurrence and metastasis. World J Gastroenterol 20: 142-147, 2014.
8. Zhu JZ, Zhou QY, Wang YM, Dai YN, Zhu J, Yu CH and Li YM: Prevalence of fatty liver disease and the economy in China: A systematic review. World J Gastroenterol 21: 5695-5706, 2015.
9. Wu Y, Zheng Q, Zou B, Yeo YH, Li X, Li J, Xie X, Feng Y, Stave CD, Zhu Q, *et al*: The epidemiology of NAFLD in Mainland China with analysis by adjusted gross regional domestic product: A meta-analysis. Hepatol Int 14: 259-269, 2020.
10. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C and Henry L: The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): A systematic review. Hepatology 77: 1335-1347, 2023.
11. Greten TF, Villanueva A, Korangy F, Ruf B, Yarchoan M, Ma L, Ruppin E and Wang XW: Biomarkers for immunotherapy of hepatocellular carcinoma. Nat Rev Clin Oncol 20: 780-798, 2023.
12. Zhang Y, Wei C, Guo CC, Bi RX, Xie J, Guan DH, Yang CH and Jiang YH: Prognostic value of microRNAs in hepatocellular carcinoma: A meta-analysis. Oncotarget 8: 107237-107257, 2017.
13. Hu Y, Setayesh T, Vaziri F, Wu X, Hwang ST, Chen X and Yvonne Wan YJ: miR-22 gene therapy treats HCC by promoting anti-tumor immunity and enhancing metabolism. Mol Ther 31: 1829-1845, 2023.
14. Song W, Zheng C, Liu M, Xu Y, Qian Y, Zhang Z, Su H, Li X, Wu H, Gong P, *et al*: TRERNA1 upregulation mediated by HBx promotes sorafenib resistance and cell proliferation in HCC via targeting NRAS by sponging miR-22-3p. Mol Ther 29: 2601-2616, 2021.
15. Menon A, Abd-Aziz N, Khalid K, Poh CL and Naidu R: miRNA: A promising therapeutic target in cancer. Int J Mol Sci 23: 11502, 2022.
16. Shukla GC, Singh J and Barik S: MicroRNAs: Processing, maturation, target recognition and regulatory functions. Mol Cell Pharmacol 3: 83-92, 2011.
17. Cui S, Chen Y, Guo Y, Wang X and Chen D: Hsa-miR-22-3p inhibits liver cancer cell EMT and cell migration/invasion by indirectly regulating SPRY2. PLoS One 18: e0281536, 2023.
18. Fan T, Wang CQ, Li XT, Yang H, Zhou J and Song YJ: MiR-22-3p suppresses cell migration and invasion by targeting PLAGL2 in breast cancer. J Coll Physicians Surg Pak 31: 937-940, 2021.
19. Wang X, Wang X, Jiang T, Zhang Z, Xie N and Yang G: MiR-22-3p suppresses NSCLC cell migration and EMT via targeting RAC1 expression. Funct Integr Genomics 23: 281, 2023.
20. Qiao H, Wang N, Guan QL, Xie P and Li XK: miR-22-3p suppresses cell proliferation and migration of gastric cancer by targeting ENO1. Altern Ther Health Med 29: 278-283, 2023.
21. Liu Y, Chen X, Cheng R, Yang F, Yu M, Wang C, Cui S, Hong Y, Liang H, Liu M, *et al*: The Jun/miR-22/HuR regulatory axis contributes to tumourigenesis in colorectal cancer. Mol Cancer 17: 11, 2018.
22. Zeng Z, Dong J, Li Y, Dong Z, Liu Z, Huang J, Wang Y, Zhen Y and Lu Y: The expression level and diagnostic value of microRNA-22 in HCC patients. Artif Cells Nanomed Biotechnol 48: 683-686, 2020.
23. Panella R, Petri A, Desai BN, Fagoonee S, Cotton CA, Nguyen PK, Lundin EM, Wagshal A, Wang DZ, Näär AM, *et al*: MicroRNA-22 is a key regulator of lipid and metabolic homeostasis. Int J Mol Sci 24: 12870, 2023.
24. Wang Y, Zhang R, Li J, Han X, Lu H, Su J, Liu Y, Tian X, Wang M, Xiong Y, *et al*: MiR-22-3p and miR-29a-3p synergistically inhibit hepatic stellate cell activation by targeting AKT3. Exp Biol Med (Maywood) 247: 1712-1731, 2022.
25. Azar S, Udi S, Drori A, Hadar R, Nemirovski A, Vemuri KV, Miller M, Sherill-Rofe D, Arad Y, Gur-Wahnon D, *et al*: Reversal of diet-induced hepatic steatosis by peripheral CB1 receptor blockade in mice is p53/miRNA-22/SIRT1/PPARα dependent. Mol Metab 42: 101087, 2020.
26. Chen J, Wu FX, Luo HL, Liu JJ, Luo T, Bai T, Li LQ and Fan XH: Berberine upregulates miR-22-3p to suppress hepatocellular carcinoma cell proliferation by targeting Sp1. Am J Transl Res 8: 4932-4941, 2016.

27. Huang W, Huang F, Zhang R and Luo H: LncRNA Neat1 expedites the progression of liver fibrosis in mice through targeting miR-148a-3p and miR-22-3p to upregulate Cyth3. Cell Cycle 20: 490-507, 2021.
28. Li H, Zhang P, Li F, Yuan G, Wang X, Zhang A and Li F: Plasma miR-22-5p, miR-132-5p, and miR-150-3p are associated with acute myocardial infarction. Biomed Res Int 2019: 5012648, 2019.
29. Wang Y, Chang W, Zhang Y, Zhang L, Ding H, Qi H, Xue S, Yu H, Hu L, Liu D, *et al*: Circulating miR-22-5p and miR-122-5p are promising novel biomarkers for diagnosis of acute myocardial infarction. J Cell Physiol 234: 4778-4786, 2019.
30. Peng WX, Koirala P and Mo YY: LncRNA-mediated regulation of cell signaling in cancer. Oncogene 36: 5661-5667, 2017.
31. Xu J, Shao T, Song M, Xie Y, Zhou J, Yin J, Ding N, Zou H, Li Y and Zhang J: MIR22HG acts as a tumor suppressor via TGFbeta/SMAD signaling and facilitates immunotherapy in colorectal cancer. Mol Cancer 19: 51, 2020.
32. Zhang W, Shi C, Xu Q, Chen X, Zhu H and Zheng B: Long non-coding RNA MIR22HG suppresses cell proliferation and promotes apoptosis in prostate cancer cells by sponging microRNA-9-3p. Bioengineered 13: 13108-13117, 2022.
33. Deng X, Ye D, Hua K, Song H, Luo Q, Munankarmy A, Liu D, Zhou B, Zheng W, Zhou X, *et al*: MIR22HG inhibits breast cancer progression by stabilizing LATS2 tumor suppressor. Cell Death Dis 12: 810, 2021.
34. Zhang L, Li C and Su X: Emerging impact of the long noncoding RNA MIR22HG on proliferation and apoptosis in multiple human cancers. J Exp Clin Cancer Res 39: 271, 2020.
35. Zhang J, Yang Y, Yang T, Liu Y, Li A, Fu S, Wu M, Pan Z and Zhou W: microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumourigenicity. Br J Cancer 103: 1215-1220, 2010.
36. Yang F, Hu Y, Liu HX and Wan YJY: MiR-22-silenced cyclin A expression in colon and liver cancer cells is regulated by bile acid receptor. J Biol Chem 290: 6507-6515, 2015.
37. Huang JN, Zhang HM, Cai JD, Wang WL and Wang P: Long noncoding RNA DSCR8 promotes the proliferation of liver cancer cells and inhibits apoptosis via the miR-22-3p/ARPC5 axis. J Cancer 14: 35-49, 2023.
38. Chen M, Hu W, Xiong CL, Qu Z, Yin CQ, Wang YH, Luo CL, Guan Q, Yuan CH and Wang FB: miR-22 targets YWHAZ to inhibit metastasis of hepatocellular carcinoma and its down-regulation predicts a poor survival. Oncotarget 7: 80751-80764, 2016.
39. Gjorgjieva M, Ay AS, Correia de Sousa M, Delangre E, Dolicka D, Sobolewski C, Maeder C, Fournier M, Sempoux C and Foti M: MiR-22 deficiency fosters hepatocellular carcinoma development in fatty liver. Cells 11: 2860, 2022.
40. Zhang L, Yang P, Wang J, Liu Q, Wang T, Wang Y and Lin F: MiR-22 regulated T cell differentiation and hepatocellular carcinoma growth by directly targeting Jarid2. Am J Cancer Res 11: 2159-2173, 2021.
41. Zhao L, Wang Y and Liu Q: Catalpol inhibits cell proliferation, invasion and migration through regulating miR-22-3p/MTA3 signalling in hepatocellular carcinoma. Exp Mol Pathol 109: 51-60, 2019.
42. Wu Y, Zhou Y, Huan L, Xu L, Shen M, Huang S and Liang L: LncRNA MIR22HG inhibits growth, migration and invasion through regulating the miR-10a-5p/NCOR2 axis in hepatocellular carcinoma cells. Cancer Sci 110: 973-984, 2019.
43. Zhang DY, Zou XJ, Cao CH, Zhang T, Lei L, Qi XL, Liu L and Wu DH: Identification and functional characterization of long non-coding RNA MIR22HG as a tumor suppressor for hepatocellular carcinoma. Theranostics 8: 3751-3765, 2018.
44. Luo LJ, Zhang LP, Duan CY, Wang B, He NN, Abulimiti P and Lin Y: The inhibition role of miR-22 in hepatocellular carcinoma cell migration and invasion via targeting CD147. Cancer Cell Int 17: 17, 2017.
45. Yim DGR, Ghosh S, Guy GR and Virshup DM: Casein kinase 1 regulates sprouty2 in FGF-ERK signaling. Oncogene 34: 474-484, 2015.
46. Quinn JJ and Chang HY: Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 17: 47-62, 2016.
47. Zhao L, Hu K, Cao J, Wang P, Li J, Zeng K, He X, Tu PF, Tong T and Han L: lncRNA miat functions as a ceRNA to upregulate sirt1 by sponging miR-22-3p in HCC cellular senescence. Aging (Albany NY) 11: 7098-7122, 2019.
48. Guo C, Zhou S, Yi W, Yang P, Li O, Liu J and Peng C: Long non-coding RNA muskelin 1 antisense RNA (MKLN1-AS) is a potential diagnostic and prognostic biomarker and therapeutic target for hepatocellular carcinoma. Exp Mol Pathol 120: 104638, 2021.
49. Pan G, Zhang J, You F, Cui T, Luo P, Wang S, Li X and Yuan Q: ETS proto-oncogene 1-activated muskelin 1 antisense RNA drives the malignant progression of hepatocellular carcinoma by targeting miR-22-3p to upregulate ETS proto-oncogene 1. Bioengineered 13: 1346-1358, 2022.
50. Guan B, Ma J, Yang Z, Yu F and Yao J: LncRNA NCK1-AS1 exerts oncogenic property in gastric cancer by targeting the miR-22-3p/BCL9 axis to activate the Wnt/β-catenin signaling. Environ Toxicol 36: 1640-1653, 2021.
51. Wang B, Wang K, Jin T, Xu Q, He Y, Cui B and Wang Y: NCK1-AS1 enhances glioma cell proliferation, radioresistance and chemoresistance via miR-22-3p/IGF1R ceRNA pathway. Biomed Pharmacother 129: 110395, 2020.
52. Zhou W, Wang J, Zhang J, Wang Y, Jiang L, Guo T, Luo B, Xu Q and Huang Y: LncRNA NCK1-AS1 aggravates hepatocellular carcinoma by the miR-22-3p/YARS axis to activate PI3K/AKT signaling. J Gastrointestin Liver Dis 31: 48-59, 2022.
53. Zhang C, Lin X, Zhao Q, Wang Y, Jiang F, Ji C, Li Y, Gao J, Li J and Shen L: YARS as an oncogenic protein that promotes gastric cancer progression through activating PI3K-Akt signaling. J Cancer Res Clin Oncol 146: 329-342, 2020.
54. Pant K, Yadav AK, Gupta P, Islam R, Saraya A and Venugopal SK: Butyrate induces ROS-mediated apoptosis by modulating miR-22/SIRT-1 pathway in hepatic cancer cells. Redox Biol 12: 340-349, 2017.
55. Yang F, Gong J, Wang G, Chen P, Yang L and Wang Z: Waltonitone inhibits proliferation of hepatoma cells and tumorigenesis via FXR-miR-22-CCNA2 signaling pathway. Oncotarget 7: 75165-75175, 2016.
56. Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, Yi P, Tang L, Pan Q, Rao S, *et al*: The cancer metabolic reprogramming and immune response. Mol Cancer 20: 28, 2021.
57. Donne R and Lujambio A: The liver cancer immune microenvironment: Therapeutic implications for hepatocellular carcinoma. Hepatology 77: 1773-1796, 2023.
58. Jhunjhunwala S, Hammer C and Delamarre L: Antigen presentation in cancer: Insights into tumour immunogenicity and immune evasion. Nat Rev Cancer 21: 298-312, 2021.
59. Kumar R, Theiss AL and Venuprasad K: RORgammat protein modifications and IL-17-mediated inflammation. Trends Immunol 42: 1037-1050, 2021.
60. Lequeux A, Noman MZ, Xiao M, Van Moer K, Hasmim M, Benoit A, Bosseler M, Viry E, Arakelian T, Berchem G, *et al*: Targeting HIF-1 alpha transcriptional activity drives cytotoxic immune effector cells into melanoma and improves combination immunotherapy. Oncogene 40: 4725-4735, 2021.
61. Togashi Y, Shitara K and Nishikawa H: Regulatory T cells in cancer immunosuppression-implications for anticancer therapy. Nat Rev Clin Oncol 16: 356-371, 2019.
62. Kang JH and Zappasodi R: Modulating Treg stability to improve cancer immunotherapy. Trends Cancer 9: 911-927, 2023.
63. Golden-Mason L and Rosen HR: Galectin-9: Diverse roles in hepatic immune homeostasis and inflammation. Hepatology 66: 271-279, 2017.
64. Sauer N, Janicka N, Szlasa W, Skinderowicz B, Kołodzińska K, Dwernicka W, Oślizło M, Kulbacka J, Novickij V and Karłowicz-Bodalska K: TIM-3 as a promising target for cancer immunotherapy in a wide range of tumors. Cancer Immunol Immunother 72: 3405-3425, 2023.
65. Zhao L, Cheng S, Fan L, Zhang B and Xu S: TIM-3: An update on immunotherapy. Int Immunopharmacol 99: 107933, 2021.
66. Yang Q, Jiang W, Zhuang C, Geng Z, Hou C, Huang D, Hu L and Wang X: microRNA-22 downregulation of galectin-9 influences lymphocyte apoptosis and tumor cell proliferation in liver cancer. Oncol Rep 34: 1771-1778, 2015.
67. Shao X, Zhu J, Shi Y, Fang H, Chen J, Zhang Y, Wang J, Jian H, Lan S, Jiang F, *et al*: Upregulated UBE4B expression correlates with poor prognosis and tumor immune infiltration in hepatocellular carcinoma. Aging (Albany NY) 14: 9632-9646, 2022.
68. McGlynn KA, Petrick JL and El-Serag HB: Epidemiology of hepatocellular carcinoma. Hepatology 73 (Suppl 1): S4-S13, 2021.
69. Qiao DD, Yang J, Lei XF, Mi GL, Li SL, Li K, Xu CQ and Yang HL: Expression of microRNA-122 and microRNA-22 in HBV-related liver cancer and the correlation with clinical features. Eur Rev Med Pharmacol Sci 21: 742-747, 2017.
70. Ke RS, Zhang K, Lv LZ, Dong YP, Pan F, Yang F, Cai QC and Jiang Y: Prognostic value and oncogene function of heterogeneous nuclear ribonucleoprotein A1 overexpression in HBV-related hepatocellular carcinoma. Int J Biol Macromol 129: 140-151, 2019.

71. Shi C and Xu X: MicroRNA-22 is down-regulated in hepatitis B virus-related hepatocellular carcinoma. Biomed Pharmacother 67: 375-380, 2013.
72. Qian C and Liu Q: FOXO3a inhibits nephroblastoma cell proliferation, migration and invasion, and induces apoptosis through downregulating the Wnt/β-catenin signaling pathway. Mol Med Rep 24: 796, 2021.
73. Tian Y, Qi P and Hu X: Downregulated FOXO3a associates with poor prognosis and promotes cell invasion and migration via WNT/β-catenin signaling in cervical carcinoma. Front Oncol 10: 903, 2020.
74. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM and Karin M: Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317: 121-124, 2007.
75. Sakurai T, He G, Matsuzawa A, Yu GY, Maeda S, Hardiman G and Karin M: Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. Cancer Cell 14: 156-165, 2008.
76. Jiang R, Deng L, Zhao L, Li X, Zhang F, Xia Y, Gao Y, Wang X and Sun B: miR-22 promotes HBV-related hepatocellular carcinoma development in males. Clin Cancer Res 17: 5593-5603, 2011.
77. Pandey DP and Picard D: miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor alpha mRNA. Mol Cell Biol 29: 3783-3790, 2009.
78. Chen S, Pu J, Bai J, Yin Y, Wu K, Wang J, Shuai X, Gao J, Tao K, Wang G and Li H: EZH2 promotes hepatocellular carcinoma progression through modulating miR-22/galectin-9 axis. J Exp Clin Cancer Res 37: 3, 2018.
79. Guo L, Hu C, Yao M and Han G: Mechanism of sorafenib resistance associated with ferroptosis in HCC. Front Pharmacol 14: 1207496, 2023.
80. Cheng Y, Takeuchi H, Sonobe Y, Jin S, Wang Y, Horiuchi H, Parajuli B, Kawanokuchi J, Mizuno T and Suzumura A: Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. J Neuroimmunol 269: 38-43, 2014.
81. Portmann S, Fahrner R, Lechleiter A, Keogh A, Overney S, Laemmle A, Mikami K, Montani M, Tschan MP, Candinas D and Stroka D: Antitumor effect of SIRT1 inhibition in human HCC tumor models in vitro and in vivo. Mol Cancer Ther 12: 499-508, 2013.
82. Jin Q, Hu H, Yan S, Jin L, Pan Y, Li X, Peng Y and Cao P: lncRNA MIR22HG-derived miR-22-5p enhances the radiosensitivity of hepatocellular carcinoma by increasing histone acetylation through the inhibition of HDAC2 activity. Front Oncol 11: 572585, 2021.
83. Yang J, Yan B, Yang L, Li H, Fan Y, Zhu F, Zheng J and Ma X: Macrocytic anemia is associated with the severity of liver impairment in patients with hepatitis B virus-related decompensated cirrhosis: A retrospective cross-sectional study. BMC Gastroenterol 18: 161, 2018.
84. Adigun OO, Yarrarapu SNS, Zubair M and Khetarpal S: Alpha-fetoprotein analysis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2024.
85. Li CQ, Huang H, Ruan SM, Hu HT, Xian MF, Xie XY, Lu MD, Kuang M, Wang Y and Chen LD: An assessment of liver lesions using a combination of CEUS LI-RADS and AFP. Abdom Radiol (NY) 47: 1311-1320, 2022.
86. Tzartzeva K and Singal AG: Testing for AFP in combination with ultrasound improves early liver cancer detection. Expert Rev Gastroenterol Hepatol 12: 947-949, 2018.
87. Zekri ARN, Youssef ASED, El-Desouky ED, Ahmed OS, Lotfy MM, Nassar AAM and Bahnassey AA: Serum microRNA panels as potential biomarkers for early detection of hepatocellular carcinoma on top of HCV infection. Tumour Biol 37: 12273-12286, 2016.
88. Xia L, Wang S, Zhang H, Yang Y, Wei J, Shi Y, Zou C, Liu J, Luo M, Huang A and Wang D: The HBx and HBc of hepatitis B virus can influence Id1 and Id3 by reducing their transcription and stability. Virus Res 284: 197973, 2020.
89. Li J, Zhang X, Chen L, Zhang Z, Zhang J, Wang W, Wu M, Shi B, Zhang X, Kozlowski M, *et al*: Circulating miR-210 and miR-22 combined with ALT predict the virological response to interferon-alpha therapy of CHB patients. Sci Rep 7: 15658, 2017.
90. Hu Q, Wang Q, Zhang Y, Tao S, Zhang X, Liu X, Li X, Jiang X, Huang C, Xu W, *et al*: Baseline serum exosome-derived miRNAs predict HBeAg seroconversion in chronic hepatitis B patients treated with peginterferon. J Med Virol 93: 4939-4948, 2021.
91. Badmus OO, Hillhouse SA, Anderson CD, Hinds TD and Stec DE: Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): Functional analysis of lipid metabolism pathways. Clin Sci (Lond) 136: 1347-1366, 2022.
92. Zhang P, Wang W, Mao M, Gao R, Shi W, Li D, Calderone R, Sui B, Tian X and Meng X: Similarities and differences: A comparative review of the molecular mechanisms and effectors of NAFLD and AFLD. Front Physiol 12: 710285, 2021.
93. Chen D, Yan Y, Wang X, Li S, Liu Y, Yu D, He Y, Deng R, Liu Y, Xu M, *et al*: Chronic alcohol exposure promotes HCC stemness and metastasis through β-catenin/miR-22-3p/TET2 axis. Aging (Albany NY) 13: 14433-14455, 2021.
94. Yang Z, Qin W, Huo J, Zhuo Q, Wang J and Wang L: MiR-22 modulates the expression of lipogenesis-related genes and promotes hepatic steatosis in vitro. FEBS Open Bio 11: 322-332, 2021.
95. Hu Y, Liu HX, Jena PK, Sheng L, Ali MR and Wan YY: miR-22 inhibition reduces hepatic steatosis via FGF21 and FGFR1 induction. JHEP Rep 2: 100093, 2020.
96. Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, Kassir R, Singhal R, Mahawar K and Ramnarain D: Non-alcoholic fatty liver disease (NAFLD): A review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord 22: 63, 2022.
97. Liu Q, Li J, Zhang W, Xiao C, Zhang S, Nian C, Li J, Su D, Chen L, Zhao Q, *et al*: Glycogen accumulation and phase separation drives liver tumor initiation. Cell 184: 5559-5576.e19, 2021.
98. Agosti P, Sabbà C and Mazzocca A: Emerging metabolic risk factors in hepatocellular carcinoma and their influence on the liver microenvironment. Biochim Biophys Acta Mol Basis Dis 1864: 607-617, 2018.
99. Hyun J, Han J, Lee C, Yoon M and Jung Y: Pathophysiological aspects of alcohol metabolism in the liver. Int J Mol Sci 22: 5717, 2021.
100. Jeon S and Carr R: Alcohol effects on hepatic lipid metabolism. J Lipid Res 61: 470-479, 2020.
101. Lu W, Li X and Luo Y: FGF21 in obesity and cancer: New insights. Cancer Lett 499: 5-13, 2021.
102. Rodríguez-Agudo R, González-Recio I, Serrano-Maciá M, Bravo M, Petrov P, Blaya D, Herranz JM, Mercado-Gómez M, Rejano-Gordillo CM, Lachiondo-Ortega S, *et al*: Anti-miR-873-5p improves alcohol-related liver disease by enhancing hepatic deacetylation via SIRT1. JHEP Rep 6: 100918, 2023.
103. Iwagami Y, Zou J, Zhang H, Cao K, Ji C, Kim M and Huang CK: Alcohol-mediated miR-34a modulates hepatocyte growth and apoptosis. J Cell Mol Med 22: 3987-3995, 2018.
104. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE and Loomba R: AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology 77: 1797-1835, 2023.
105. No authors listed: The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American association for the study of liver diseases. Clin Liver Dis (Hoboken) 11: 81, 2018.
106. Castano C, Novials A and Párrizas M: Exosomes from short-term high-fat or high-sucrose fed mice induce hepatic steatosis through different pathways. Cells 12: 169, 2022.
107. Thibonnier M and Esau C: Metabolic benefits of MicroRNA-22 inhibition. Nucleic Acid Ther 30: 104-116, 2020.
108. Thibonnier M, Esau C, Ghosh S, Wargent E and Stocker C: Metabolic and energetic benefits of microRNA-22 inhibition. BMJ Open Diabetes Res Care 8: e001478, 2020.
109. Gjorgjieva M, Sobolewski C, Ay AS, Abegg D, Correia de Sousa M, Portius D, Berthou F, Fournier M, Maeder C, Rantakari P, *et al*: Genetic ablation of MiR-22 fosters diet-induced obesity and NAFLD development. J Pers Med 10: 170, 2020.
110. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A and Nader F: The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol 71: 793-801, 2019.
111. Agbu P and Carthew RW: MicroRNA-mediated regulation of glucose and lipid metabolism. Nat Rev Mol Cell Biol 22: 425-438, 2021.
112. Kaur K, Vig S, Srivastava R, Mishra A, Singh VP, Srivastava AK and Datta M: Elevated hepatic miR-22-3p expression impairs gluconeogenesis by silencing the Wnt-responsive transcription factor Tcf7. Diabetes 64: 3659-3669, 2015.

113. Petito G, Cioffi F, Silvestri E, De Matteis R, Lattanzi D, de Lange P, Lombardi A, Moreno M, Goglia F, Lanni A and Senese R: 3,5-Diiodo-L-thyronine (T2) administration affects visceral adipose tissue inflammatory state in rats receiving long-lasting high-fat diet. Front Endocrinol (Lausanne) 12: 703170, 2021.
114. Silvestri E, Cioffi F, De Matteis R, Senese R, de Lange P, Coppola M, Salzano AM, Scaloni A, Ceccarelli M, Goglia F, *et al*: 3,5-Diiodo-L-thyronine affects structural and metabolic features of skeletal muscle mitochondria in high-fat-diet fed rats producing a co-adaptation to the glycolytic fiber phenotype. Front Physiol 9: 194, 2018.
115. Senese R, Cioffi F, Petito G, de Lange P, Russo A, Goglia F, Lanni A and Potenza N: miR-22-3p is involved in gluconeogenic pathway modulated by 3,5-diiodo-L-thyronine (T2). Sci Rep 9: 16645, 2019.
116. Zhao T, Wang J, He A, Wang S, Chen Y, Lu J, Lv J, Li S, Wang J, Qian M, *et al*: Mebhydrolin ameliorates glucose homeostasis in type 2 diabetic mice by functioning as a selective FXR antagonist. Metabolism 119: 154771, 2021.
117. Cohen-Naftaly M and Friedman SL: Current status of novel antifibrotic therapies in patients with chronic liver disease. Therap Adv Gastroenterol 4: 391-417, 2011.
118. Elpek GÖ: Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol 20: 7260-7276, 2014.
119. Higashi T, Friedman SL and Hoshida Y: Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev 121: 27-42, 2017.
120. Ezhilarasan D: MicroRNA interplay between hepatic stellate cell quiescence and activation. Eur J Pharmacol 885: 173507, 2020.
121. Riaz F, Chen Q, Lu K, Osoro EK, Wu L, Feng L, Zhao R, Yang L, Zhou Y, He Y, *et al*: Inhibition of miR-188-5p alleviates hepatic fibrosis by significantly reducing the activation and proliferation of HSCs through PTEN/PI3K/AKT pathway. J Cell Mol Med 25: 4073-4087, 2021.
122. Chen X, Zhu S, Chen SY, Wang JN, Sun LJ, Tao SM, Li XF, Li HD, Sun YY, Xu CH, *et al*: miR-301a-3p promotes hepatic stellate cells activation and liver fibrogenesis via regulating PTEN/PDGFR-β. Int Immunopharmacol 110: 109034, 2022.
123. Ju A, Shen Y and Yue A: Circ_0011232 contributes to hepatocellular carcinoma progression through miR-503-5p/AKT3 axis. Hepatol Res 52: 532-545, 2022.
124. Zheng Y, Cai B, Li X, Li D and Yin G: MiR-125b-5p and miR-181b-5p inhibit keratinocyte proliferation in skin by targeting Akt3. Eur J Pharmacol 862: 172659, 2019.
125. Zhang Y, Wang F, Chen G, He R and Yang L: LncRNA MALAT1 promotes osteoarthritis by modulating miR-150-5p/AKT3 axis. Cell Biosci 9: 54, 2019.
126. Abdullah AS, Sayed IETE, El-Torgoman AMA, Kalam A, Wageh S and Kamel MA: Green synthesis of silymarin-chitosan nanoparticles as a new nano formulation with enhanced anti-fibrotic effects against liver fibrosis. Int J Mol Sci 23: 5420, 2022.
127. Abdullah AS, El Sayed IET, El-Torgoman AMA, Alghamdi NA, Ullah S, Wageh S and Kamel MA: Preparation and characterization of silymarin-conjugated gold nanoparticles with enhanced anti-fibrotic therapeutic effects against hepatic fibrosis in rats: Role of MicroRNAs as molecular targets. Biomedicines 9: 1767, 2021.
128. Tsuchida T, Lee YA, Fujiwara N, Ybanez M, Allen B, Martins S, Fiel MI, Goossens N, Chou HI, Hoshida Y and Friedman SL: A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. J Hepatol 69: 385-395, 2018.
129. Ji D, Li B, Shao Q, Li F, Li Z and Chen G: MiR-22 suppresses BMP7 in the development of cirrhosis. Cell Physiol Biochem 36: 1026-1036, 2015.
130. Onakpoya IJ, Heneghan CJ and Aronson JK: Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: A systematic review of the world literature. BMC Med 14: 10, 2016.
131. Li X, Tang J and Mao Y: Incidence and risk factors of drug-induced liver injury. Liver Int 42: 1999-2014, 2022.
132. Kleiner DE: Drug-induced liver injury: The hepatic pathologist's approach. Gastroenterol Clin North Am 46: 273-296, 2017.
133. Kleiner DE, Chalasani NP, Lee WM, Fontana RJ, Bonkovsky HL, Watkins PB, Hayashi PH, Davern TJ, Navarro V, Reddy R, *et al*: Hepatic histological findings in suspected drug-induced liver injury: Systematic evaluation and clinical associations. Hepatology 59: 661-670, 2014.
134. Yang Z, Wu W, Ou P, Wu M, Zeng F, Zhou B and Wu S: MiR-122-5p knockdown protects against APAP-mediated liver injury through up-regulating NDRG3. Mol Cell Biochem 476: 1257-1267, 2021.
135. Vliegenthart ADB, Berends C, Potter CMJ, Kersaudy-Kerhoas M and Dear JW: MicroRNA-122 can be measured in capillary blood which facilitates point-of-care testing for drug-induced liver injury. Br J Clin Pharmacol 83: 2027-2033, 2017.
136. Liu Y, Chen H, Hao J, Li Z, Hou T and Hao H: Characterization and functional prediction of the microRNAs differentially expressed in a mouse model of concanavalin A-induced autoimmune hepatitis. Int J Med Sci 17: 2312-2327, 2020.
137. López-Riera M, Conde I, Tolosa L, Zaragoza A, Castell JV, Gómez-Lechón MJ and Jover R: New microRNA biomarkers for drug-induced steatosis and their potential to predict the contribution of drugs to non-alcoholic fatty liver disease. Front Pharmacol 8: 3, 2017.
138. Amacher DE and Chalasani N: Drug-induced hepatic steatosis. Semin Liver Dis 34: 205-214, 2014.
139. Allard J, Le Guillou D, Begriche K and Fromenty B: Drug-induced liver injury in obesity and nonalcoholic fatty liver disease. Adv Pharmacol 85: 75-107, 2019.